

42

sau96I
haeIII
asul
sau96I
nlaIV
hg1JI
eco0109I
bsp1286
banII
asul
nlaIV

1 mnII aval apal mnII
-18 mboII mnII eco0109I tth111I
GGA CTT GTC TTC CTC GTC CTG CTG TTC CTC GGG GCC CTC GGA CTG
Gly Leu Val Phe Leu Val Leu Leu Phe Leu Gly Ala Leu Gly Leu

haeIII
eacl
cfrI

hinPI
hhai

46 TGT CTG GCT GGC CGT AGG AGA AGG AGT GTT CAG TGG TGC GCC GTA TCC
-3 Cys Leu Ala Gly Arg Arg Arg Ser Val Gln Trp Cys Ala Val Ser

haeIII
mnII
aval haeI

94 CAA CCC GAG GCC ACA AAA TGC TTC CAA TGG CAA AGG AAT ATG AGA AAA
14 Gln Pro Glu Ala Thr Lys Cys Phe Gln Trp Gln Arg Asn Met Arg Lys

mnII	fnu4HI		
sau96I	bbvI	pleI	
haeIII	alul	hinfl	bsrI
asul	pvuII	bsmal	fokI

142 GTG CTG GGC CCT CCT GTC AGC TGC ATA AAG AGA GAC TCC CCC ATC CAG
30 Val Arg Gly Pro Pro Val Ser Cys Ile Lys Arg Asp Ser Pro Ile Gln

haeIII	haeIII
haeI	
scrFI	scrFI
ecoRII	sau96I
bstNI	asul sfaNI

190 TGT ATC CAG GCC ATT GCG GAA AAC AGG GGC GAT GCT GTG ACC CTT GAT
46 Cys Ile Gln Ala Ile Ala Glu Asn Arg Ala Asp Ala Val Thr Leu Asp

sau96I	
nlaIV	
scrFI	
ecoRII	
bstNI	
haeIII	
stuI	haeIII
mnII	haeI
	asul

238 GGT GGT TTC ATA TAC GAG GCA GGC CTG GCC CCC TAC AAA CTG CGA CCT
62 Gly Gly Phe Ile Tyr Glu Ala Gly Leu Ala Pro Tyr Lys Leu Arg Pro

sau96I
avaII
asuI
fnu4HI accI nlaIV
286 GTA GCG GCG GAA GTC TAC GGG ACC GAA AGA CAG CCA CGA ACT CAC TAT
78 Val Ala Ala Glu Val Tyr Gly Thr Glu Arg Gln Pro Arg Thr His Tyr

fnu4HI
mbolI bbvI aluI
hphI fnu4HI aluI pvuII
334 TAT GCC GTG GCT GTG GTG AAG AAG GGC GGC AGC TTT CAG CTG AAC GAA
94 Tyr Ala Val Ala Val Val Lys Lys Gly Gly Ser Phe Gln Leu Asn Glu

haeIII sau96I
stuI avaII
bglI haeI asuI fokI
382 CTG CAA GGT CTG AAG TCC TGC CAC ACA GGC CTT CGC AGG ACC GCT GGA
110 Leu Gln Gly Leu Lys Ser Cys His Thr Gly Leu Arg Arg Thr Ala Gly

sau96I
avaII
asuI
nlaIV
430 TGG AAT GTC CCT ACA GGG ACA CTT CGT CCA TTC TTG AAT TGG ACG GGT
126 Trp Asn Val Pro Thr Gly Thr Leu Arg Pro Phe Leu Asn Trp Thr Gly

hg1JI aluI
bsp1286 fnu4HI
banII bbvI ddeI aluI
ddeI mnI pvuII mboII pvuII
478 CCA CCT GAG CCC ATT GAG GCA GCT GTG CAG TTC TCA GCC AGC TGT
142 Pro Pro Glu Pro Ile Glu Ala Ala Val Gln Phe Phe Ser Ala Ser Cys

mspI
hpaII
scrFI
ncII
cauII
526 GTT CCC GGT GCA GAT AAA GGA CAG TTC CCC AAC CTG TGT CGC CTG TGT
158 Val Pro Gln Ala Asp Lys Gly Gln Phe Pro Asn Leu Cys Arg Leu Cys

nlaIV
scrFI
ecoRII
mnI bstNI rsal
574 GCG GGG ACA GGG GAA AAC AAA TGT GCC TTC TCC TCC CAG GAA CCG TAC
174 Ala Gly Thr Gly Glu Asn Lys Cys Ala Phe Ser Ser Gln Glu Pro Tyr

FIG.-1

nlaIV
 hgiCI
 aluI banI ddeI bsmAI bsmAI
 622 TTC AGC TAC TCT GGT GCC TTC AAG TGT CTG AGA GAC GGG GCT GGA GAC
 190 Phe Ser Tyr Ser Gly Ala Phe Lys Cys Leu Arg Asp Gly Ala Gly Asp

sau96I
 avall
 asuI
 ppuMI
 hgIAI eco0109I
 bsp1286 mnII mnII
 670 GTG GCT TTT ATC AGA GAG AGC ACA GTG TTT GAG GAC CTG TCA GAC GAG
 206 Val Ala Phe Ile Arg Glu Ser Thr Val Phe Glu Asp Leu Ser Asp Glu

718 GCT GAA AGG GAC GAG TAT GAG TTA CTC TGC CCA GAC AAC ACT CGG AAG
 222 Ala Glu Arg Asp Glu Tyr Glu Leu Leu Cys Pro Asp Asn Thr Arg Lys

scrFI
 ncII
 mspl
 hpaII
 cauII
 xmaI sau96I
 smaI nlaIV
 scrFI
 ncII avall
 cauII
 avaI asuI
 sau96I ppuMI
 haeIII nlaIV
 asuI eco0109I nlaIII
 bsrI
 766 CCA GTG GAC AAG TTC AAA GAC TGC CAT CTG GCC CGG GTC CCT TCT CAT
 238 Pro Val Asp Lys Phe Lys Asp Cys His Leu Ala Arg Val Pro Ser His

sfaNI
 fokI mbalI
 bglI draIII mnII hinfl
 814 GCC GTT GTG GCA CGA AGT GTG AAT GGC AAG GAG GAT GCC ATC TGG AAT
 254 Ala Val Val Ala Arg Ser Val Asn Gly Lys Glu Asp Ala Ile Trp Asn

scrFI
 ecoRII
 bstNI hphI
 862 CTT CTC CGC CAG GCA CAG GAA AAG TTT GGA AAG GAC AAG TCA CCG AAA
 270 Leu Leu Arg Gln Ala Gln Glu Lys Phe Gly Lys Asp Lys Ser Pro Lys

sau3AI
 mboI
 dpnI
 xholI
 aluI
 bstXI nlaIV bglII
 910 TTC CAG CTC TTT GGC TCC CCT AGT GGG CAG AAA GAT CTG CTG TTC AAG
 286 Phe Gln Leu Phe Gly Ser Pro Ser Gly Gln Lys Asp Leu Leu Phe Lys

nlaIV
 hgiCI
 pleI mnII bsp1286 mnII
 hinFI taqI banI aval hinFI
 958 GAC TCT GCC ATT GGG TTT TCG AGG GTG CCC CCG AGG ATA GAT TCT GGG
 302 Asp Ser Ala Ile Gly Phe Ser Arg Val Pro Pro Arg Ile Asp Ser Gly

mspl
 styI hpaII
 rsal nlaIV fokI mnII
 1006 CTG TAC CTT GGC TCC GGC TAC TTC ACT GCC ATC CAG AAC TTG AGG AAA
 318 Leu Tyr Leu Gly Ser Gly Tyr Phe Thr Ala Ile Gln Asn Leu Arg Lys

mspl
 hpaII thal
 scrFI fnuDII
 nciI bstUI
 mnII fnu4HI hinPI
 mnII bbvI cauII hhaI
 1054 AGT GAG GAG GAA GTG GCT GCC CGG CGT GCG CGG GTC GTG TGG TGT GCG
 344 Ser Glu Glu Glu Val Ala Ala Arg Arg Ala Arg Val Val Trp Cys Ala

hinPI
 mstI
 fspI
 fnu4HI
 aluI hhaI bstXI
 alwNI bbvI bsRI
 1102 GTG GGC GAG CAG GAG CTG CGC AAG TGT AAC CAG TGG AGT GGC TTG AGC
 350 Val Gly Glu Gln Glu Leu Arg Lys Cys Asn Gln Trp Ser Gly Leu Ser

fnu4HI mnII
 bbvI bspMI mnII haellII mnII sfaNI
 1150 GAA GGC AGC GTG ACC TGC TCC TCG GCC TCC ACC ACA GAG GAC TGC ATC
 366 Glu Gly Ser Val Thr Cys Ser Ser Ala Ser Thr Thr Glu Asp Cys Ile

scrFI
 ecoRII
 bstNI aluI sfaNI nlaIII fokI mnII
 1198 GCC CTG GTG CTG AAA GGA GAA GCT GAT GCC ATG AGT TTG GAT GGA GGA
 382 Ala Leu Val Leu Lys Gly Glu Ala Asp Ala Met Ser Leu Asp Gly Gly

hphI bsp1286
 1582 GAC GAG CAG GGT GAG AAT AAG TGC GTG CCC AAC AGC AAT GAG AGA TAC
 510 Asp Glu Gln Gly Glu Asn Lys Cys Val Pro Asn Ser Asn Glu Arg Tyr

nlaIV
 hgiCI
 banI scrFI
 mspI ecoRII
 bsrI hpall bstNI ddeI bsmI bsmal
 1630 TAC GGC TAC ACT GGG GCT TTC CGG TGC CTG GCT GAG AAT GCT GGA GAC
 526 Tyr Gly Tyr Thr Gly Ala Phe Arg Cys Leu Ala Glu Asn Ala Gly Asp

1678 GTT GCA TTT GTG AAA GAT GTC ACT GTC TTG CAG AAC ACT GAT GGA AAT
 542 Val Ala Phe Val Lys Asp Val Thr Val Leu Gln Asn Thr Asp Gly Asn

fnu4HI
 bbvI
 hinPI
 hhaI
 mnII nlaIII ddeI aluI
 1726 AAC AAT GAG GCA TGG GCT AAG GAT TTG AAG CTG GCA GAC TTT GCG CTG
 558 Asn Asn Glu Ala Trp Ala Lys Asp Leu Lys Leu Ala Asp Phe Ala Leu

taqI fnu4HI
 mnII bbvI
 bglI ddeI aluI
 1774 CTG TGC CTC GAT GGC AAA CGG AAG CCT GTG ACT GAG GCT AGA AGC TGC
 574 Leu Cys Leu Asp Gly Lys Arg Lys Pro Val Thr Glu Ala Arg Ser Cys

sau96I fnu4HI
 nlaIV
 nlaIII
 styI haeIII
 ncoI asuI hinFI nlaIII bsmal fokI
 1822 CAT CTT GCC ATG GCC CCG AAT CAT GCC GTG GTG TCT CGG ATG GAT AAG
 590 His Leu Ala Met Ala Pro Asn His Ala Val Val Ser Arg Met Asp Lys

fnu4HI
 ecoNI alwNI bbvI
 1870 GTG GAA CGC CTG AAA CAG GTG CTG CTC CAC CAA CAG GCT AAA TTT GGG
 606 Val Glu Arg Leu Lys Gln Val Leu Leu His Gln Gln Ala Lys Phe Gly

sau3AI
 mboI mspI
 dpnI hpall
 xholI scrFI
 bstYI ncII
 alwI cauII bsrI
 1919 AGA AAT GGA TCT GAC TGC CCG GAC AAG TTT TGC TTA TTC CAG TCT GAA
 622 Arg Asn Gly Ser Asp Cys Pro Asp Lys Phe Cys Leu Phe Gln Ser Glu

haeIII
 haeI
 eaeI styl
 ddeI cfrI pleI ncol
 draIII bali hinfl
 1966 ACC AAA AAC CTT CTG TTC AAT GAC AAC ACT GAG TGT CTG GCC AGA CTC
 638 Thr Lys Asn Leu Leu Phe Asn Asp Asn Thr Glu Cys Leu Ala Arg Leu

 sau96I
 avaiI
 asuI
 nlaIII ndeI sspI nlaIV
 2014 CAT GGC AAA ACA ACA TAT GAA AAA TAT TTG GGA CCA CAG TAT GTC GCA
 654 His Gly Lys Thr Thr Tyr Glu Lys Tyr Leu Gly Pro Gln Tyr Val Ala

 scrFI
 ecoRII
 bstNI
 hgIAI
 bsp1286 mnII mnII
 2062 GGC ATT ACT AAT CGT AAA AAG TGC TCA ACC TCC CCC CTC CTG GAA GCC
 670 Gly Ile Thr Asn Leu Lys Cys Ser Thr Ser Pro Leu Leu Glu Ala

 ddeI
 mstII
 mnII sau96I
 eco81I mbolI haeIII
 ecoRI bsu36I mbolI asuI aluI
 2110 TGT GAA TTC CTC AGG AAG TAA AACCGAAGAA GATGGGCCAG CTCCCCAAGA
 685 Cys Glu Phe Leu Arg Lys DC*

 styI
 haeIII
 sau96I
 mbolI scrFI asuI
 earI ecoRII nlaIV
 aluI bstNI eco0109I nlaIV
 2161 AAGCCTCAGC CATTCACTGC CCCCCAGCTCT TCTCCCCAGG TGTGTTGGGG CCTTGGCTCC

 ecoNI fokI ddeI
 2221 CCTGCTGAAG GTGGGGATTG CCCATCCATC TGCTTACAAT TCCCTGCTGT CGTCTTAGCA

 2281 AGAAGTAAAA TGAGAAATT TGTTGATATT CAAAAAAA

 >LENGTH: 2319

FIG.-1

1 GACTCTTAAAGG GGCCTTGAGA CCTAGTGGAGA GAGAAAGAAC ATTCGAGGAG CCAGGGAGAAA CCAAGGACAGG TGAGGTGCAAG GCTGGCTTTC CTCCTGGAGC
 101 GCGGTGTTGA GTCCCTGTCCT GCTCTAGGGC TTTTCGGAGC CTGGATCTCTC AAGGAACAAG TAGACCTGGC CGCGGGGAGT GGGGAGGGAA GGGGTGCTA
 201 TTGGGCAACA GGGGGCAAAA GCCCCTGAATA AAGGGGCCAA GGGGAGGGCA CGCTTGAGAG CCCTTCGTTG CCAAGTCGCC TCCAGACCAC AGAC ATG AAA CTT
 -19 M K L

304 GTC TTC CTC GTC CTG CTG TIC GGC CTC GGA CTG TGT CGT GCT GGC CGT AGG AGA AGG AGT GTT CAG TGG TGC GCC GIA TCC
 -16 V F L V L L F L G A L G L C L A G R R S V Q W C A V S

391 CAA CCC GAG GCC ACA AAA TGC TTC CAA TGG CAA AGG AAT ATG AGA AAA GTG CGT GGC CCT CCT GTC AGC ATA AAG AGA GAC TCC
 14 Q P E A T K C F Q W Q R N M R K V R G P P V S C I K R D S

478 CCC ATC CAG TGT ATC CAG GCC ATT GCG GAA AAC AGG GCC GAT GCT GTG ACC CCT GAT GGT TIC ATA TAC GAG GCA GGC CTG GCC
 43 P I Q C 1 Q A 1 A E N R A D A V T L D G G F I Y E A G L A

565 CCC TAC AAA CTG CGA CCT GTA CGG GCG GAA GTC TAC GGG ACC GAA AGA CAG CCA CGA ACT CAC TAT TAT GCC GTG GTG GAG
 72 P Y K L R P V A E V Y G T E R Q P R T H Y Y A V A V V K

652 AAG GGC GGC AGC TTT CAG CTG AAC GAA CTG CAA GGT CTG AAG TCC TGC CAC ACA GGC CTT CGC AGG ACC GCT GGA TGG AAT GTC CCT
 101 K G S F Q L N E L Q G L K S C H T G L R R T A G W N V P

739 ACA GGG ACA CTT CGT CCA TTC TTG AAT TGG ACC GGT CCA CCT GAG GCA GCT GTG GCC AGG TTC TCC TCA GCC AGC GT
 130 T G T L R P F L N V T G P P E P I E A A V A R F F S A S C

826 GTT CCC GGT GCA GAT AAA GGA CAG TTC CCC AAC CTG TGT GGC CTG TGT GGC GGG ACA GGG GAA AAC AAA TGT GCC TTC TCC CAG
 159 V P G A D K G Q F P N L C R L C A G T G E N K C A F S S Q

913 GAA CCG TAC TTC AGC TAC TCT GGT GCC TTC AAG TGT CTG AGA GAC GGG GCT GGA GAC GTG GCT TTT ATC AGA GAG AGC ACA GTG TTT
 188 E P Y F S Y S G A F K C L R D G A G D V A F I R E S T V F

1000 GAG GAC CTG TCA GAC GAG GCT GAA AGG GAC GAG TAT GAG TTA CTC TGC CCA GAC AAC ACT CGG AAG CCA GTG GAC AAG TTC AAA GAC
 217 E D L S D E A E R D E Y E L L C P D N T R K P V D K F K D

1087 TGC CAT CTG GCG CGG GTC CCT TCT CAT GCC GTT GTG GCA CGA AGT GTG AAT GGC AAG GAG GAT GCC ATC TGG AAT CTT CTC CGC CAG
 246 C H L A R V P S H A V V A R S V N G K E D A I W N L R Q

1174 GCA CAG GAA AAG TTT GGA AAG GAC AAG TCA CGG AAA TTC CAG CTC TTT GGC TCC CCT AGT GGG CAG AAA GAT CTG CTG TTC AAG GAC
 275 A Q E K F G K D K S P K F Q L F G S P S G Q K D L L F K D

1261 TCT GCC ATT GGG TTT TCG AGG GTG CCC CGG AGG ATA GAT TCT GGG CTG TAC CTT GGC TAC TTC ACT GCC ATC GAG AAC TIG
 304 S A 1 G F S R V P P R I D S G L Y L G S G Y F T A 1 Q N L

1348 AGG AAA ATG GAG GAG GAA GTG GCT GCC CGG CGT GCC CGG GTG TGG TGT GCC GAG GAG CGC CGC AAG TGT AAC CAG
 333 R K S E E V A R R A R V V W C A V G E Q E L R K C N Q

FIG.-2

1435 TGG AGT GGC TTG ACC GGC GAA TCC TCG TCC ACC TGC ACC GAC TGC ATC GCC CTC GTG CTG AAA GGA GAA GCT
 362 W S G L S E G S V T C S A S T T E D C I A L V L K G E A
 1522 GAT GCC ATG AGT TTG GAT GGA GGA TAT GTG TAC ACT GCA TGC AAA TGT GGT CCT GTC CTG GCA GAG AAC TAC AAA TCC CAA
 391 D A M S L D G Y V T A C K C G L V P V L A E N Y K S Q
 1609 CAA AGC AGT GAC CCT GAT AAC TGT GTG GAT AGA CCT GTG GAA TAT CTT GCT GTG GCG GTG GTT AGG AGA TCA GAC ACT AGC
 420 Q S D P D P N C V D R P V E G Y L A V A V R R S D T S
 1696 CTT ACC TGG AAC TCT GTG AAA GGC AAG AAG TCC TGC CAC ACC GCC GTG GAC AGG ACT GCA GGC TGG ATT ATC CCC ATG GCC CTG CTC
 449 L T W N S V K G K S C N T A V D R T A G W N I P M Q L L
 1783 TTC AAC CAG ACG GGC TCC TGC AAA TTT GAT GAA TAT TTC AGT CAA AGC TGT GCC CCT GGG TCT GAC CCG AGA TCT AAT CTC TGT GCT
 478 F N Q T G S C K F D E Y F S Q S C A P G S D P R S N L C A
 1870 CTG TGT ATT GGC GAC GAG GGT GAG AAT AAG TGC GTG CCC AAC AGC GAG AGA TAC TAC GGC TAC ACT GGG GCT TTC CGG TGC
 507 L C I G D E Q G E N K C V P N S N E R Y Y G Y T G A F R C
 1957 CTG GCT GAG AAT GCT GGA GAC GTT GCA ATT GTG AAA GAT GTC TTG CAG AAC ACT GAT GGA ATT AAC AAT GAG GCA TGG GCT
 536 L A E N A G D V A F V K D V T V L Q N T D G N N E A W A
 2044 AAG GAT TTG AAC CTG GCA GAC TTT GCG CTG TGC CTC GAT GGC AAA CGG AAG CCT GTG ACT GAC GCT AGA AGC TGC CAT CTT GCC
 565 K D L K L A D F A L L C L D G K R K P V T E A R S C H L A
 2131 ATG GCC CCG AAT CAT GCC GTG GTG TCT GGG ATG GAT AAG GTG GAA CGG CTG AAA CAG GTG CTG CTC CAC CAA CAG GCT AAA TTT GGG
 594 M A P N H A V S R M D K V E R L K Q V L L H Q Q A K F G
 2218 AGA AAT GGA TCT GAC TGC CCC GAC AAG TTT TGC TTA TTC CAG TCT GAA ACC AAA AAC CTT CTG TTC ATT GAC AAC ACT GAG TGT CTG
 623 R N G S D C P D K F C L F Q S E T K N L F N D N T E C L
 2305 GCC AGA CTC CAT GGC AAA ACA ACA TAT GAA AAA TAT TTG GGA CCA CAG TAT GTC GCA GGC ATT ACT ATT CTG AAA AAG TGC TCA ACC
 652 A R L H G K T T Y E K Y L G P Q Y V A G I T N L K K C S T
 2392 TCC CCC CTC CTG GAA GCC TGT GAA TTC CTC AGG AAG TAA AACCGAAGAA GATGGGCCAG CTCCCCAAGA AAAGCTTCAAG CATTCACTGC CCCAGCTCT
 681 S P L E A C E F L R K O
 2491 TCTCCCCAGG TGTGTGGGG CCTTGCTCC CCTTGCTGAAG GTGGGGATG CCCATCCATC TGGCTTACAAT TCCCCTGGCTGT CGCTCTTAACTT CAAAAAAA
 2591 TGAAATTTC TGTGTATT CAAAAAA

FIG.-2

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RESTRICTION MAP OF 5' - FLANKING REGION OF α S1 CASEIN GENE

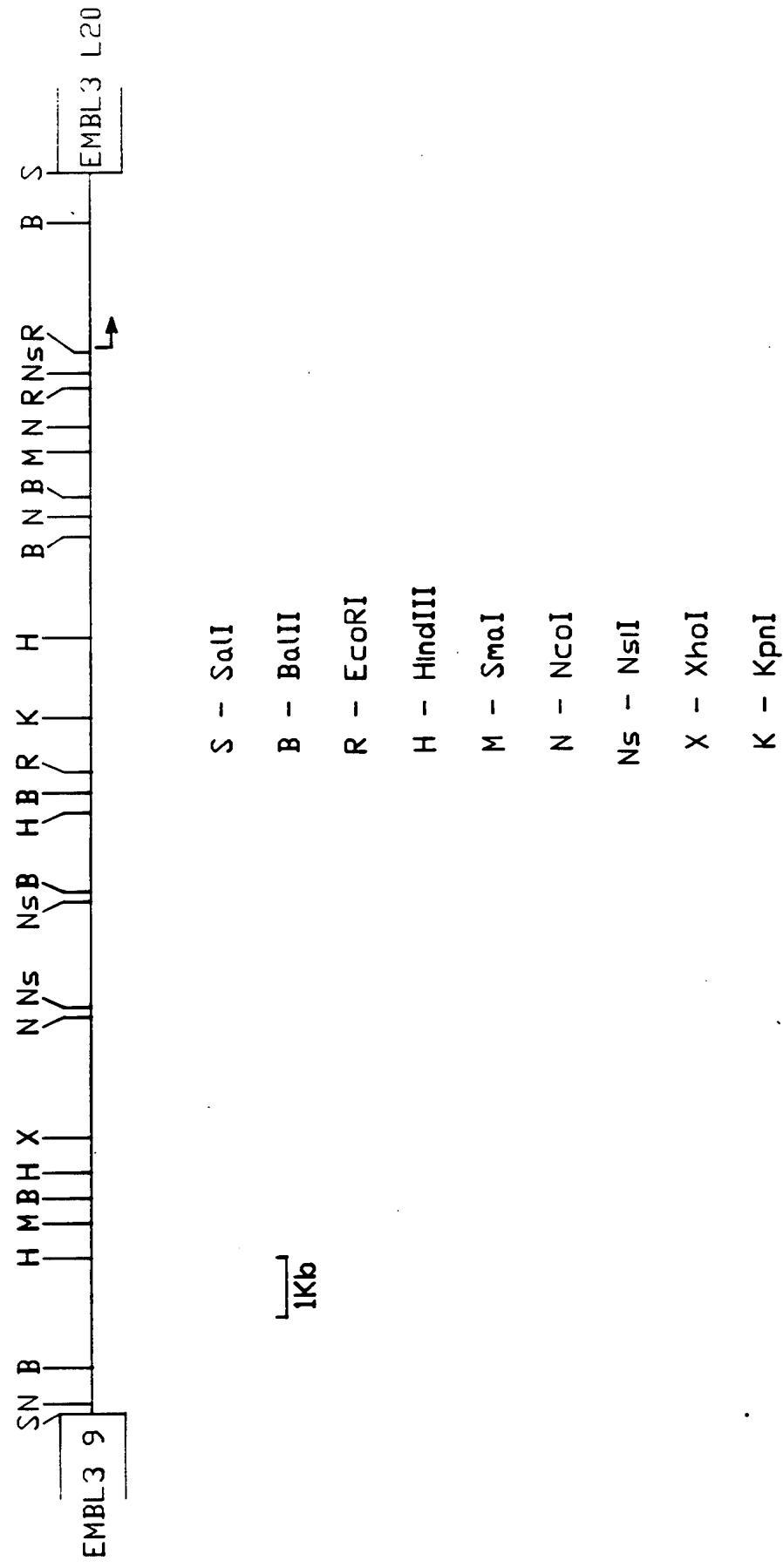
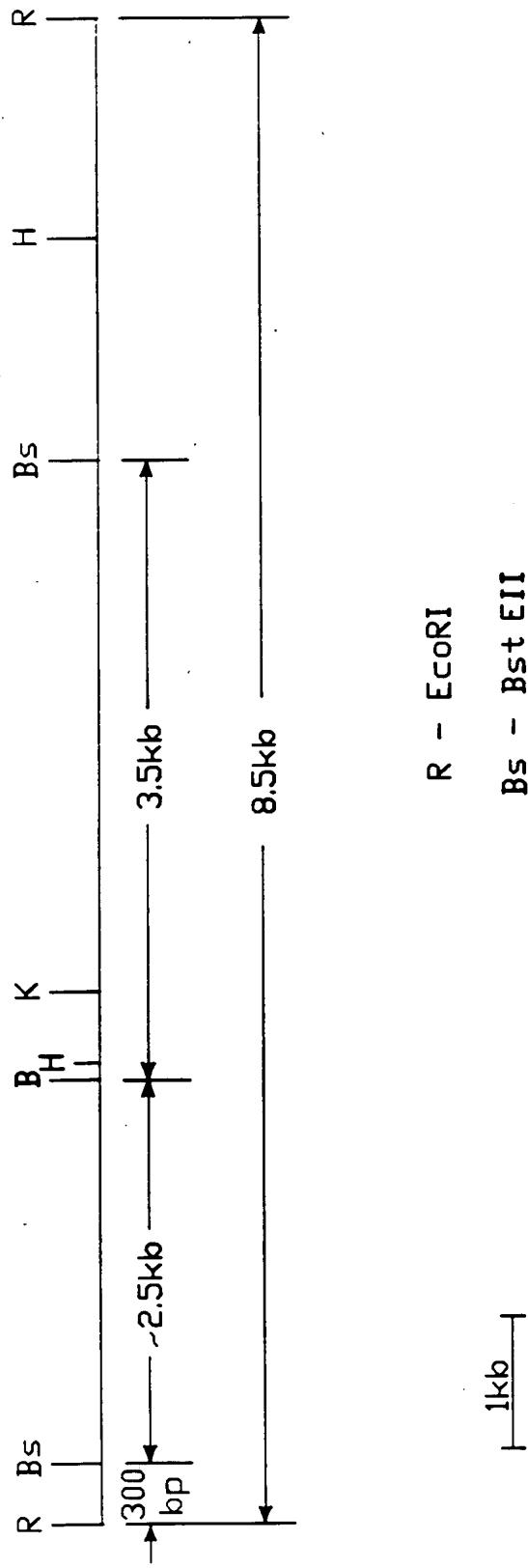


FIG.-3

RESTRICTION MAP OF 3' FLANKING
REGION OF α S1 CASEIN GENE



R - EcoRI

B_s - BstEII

B - BamHI

H - HindIII

K - KpnI

FIG.-4

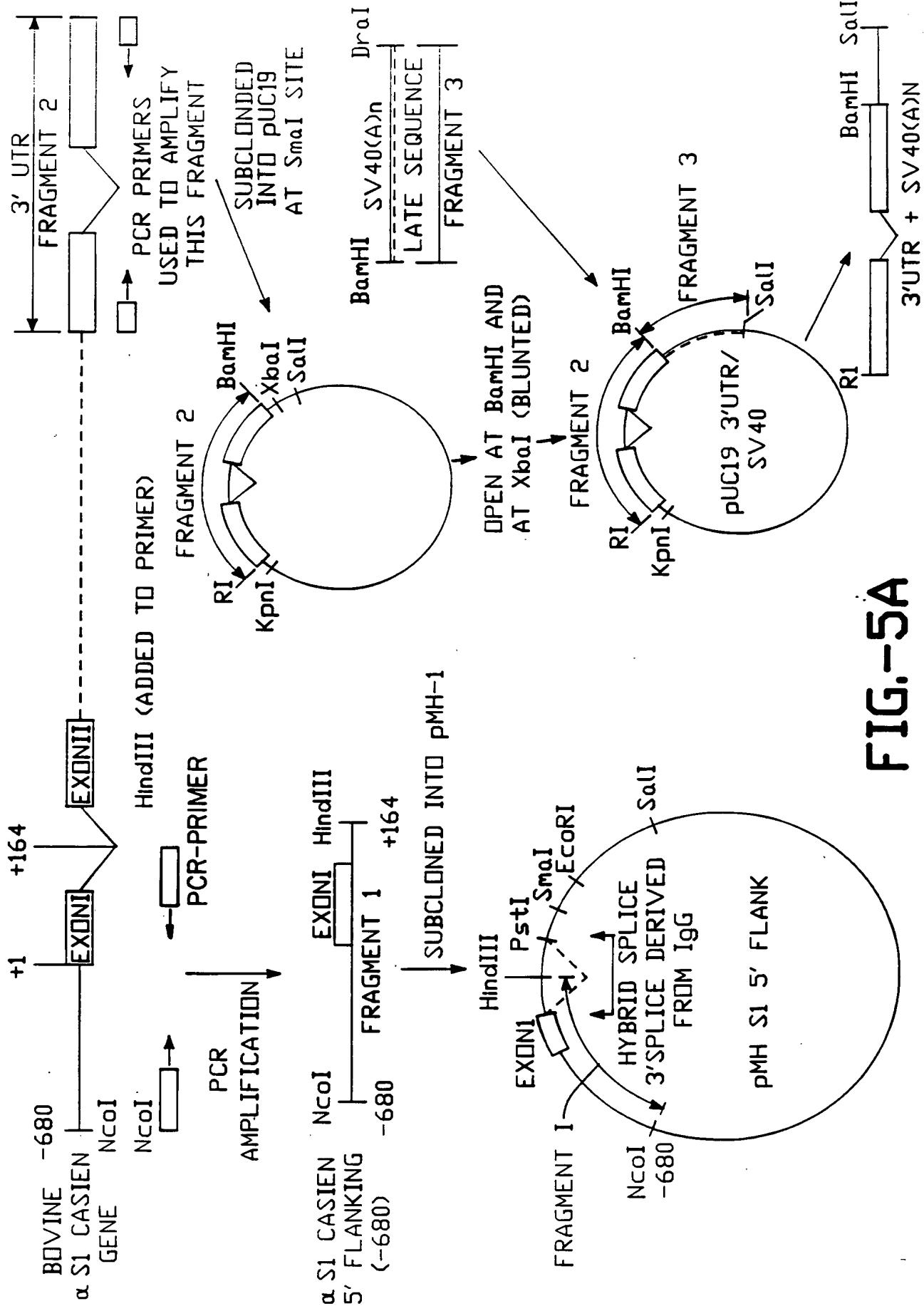
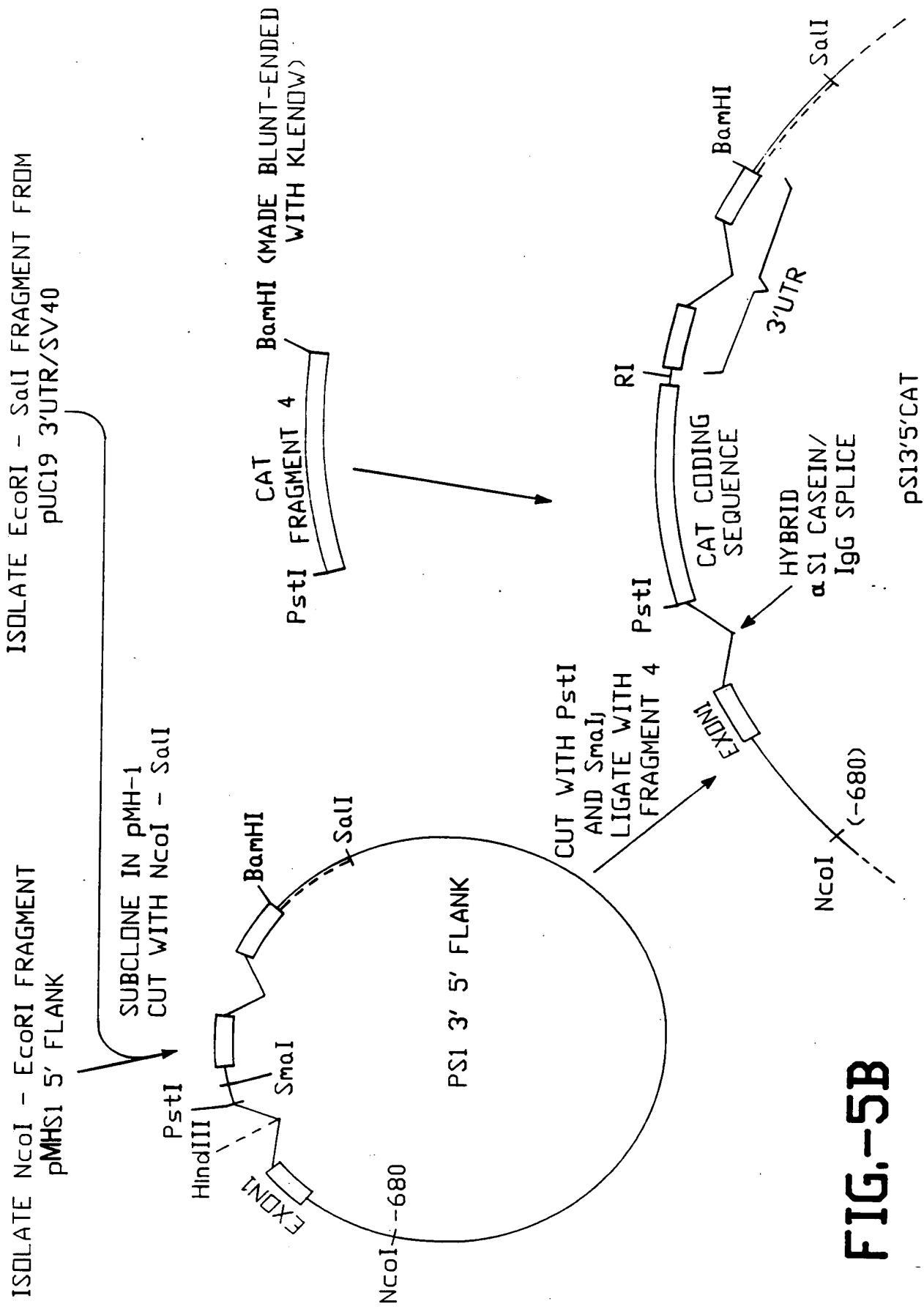


FIG.-5A



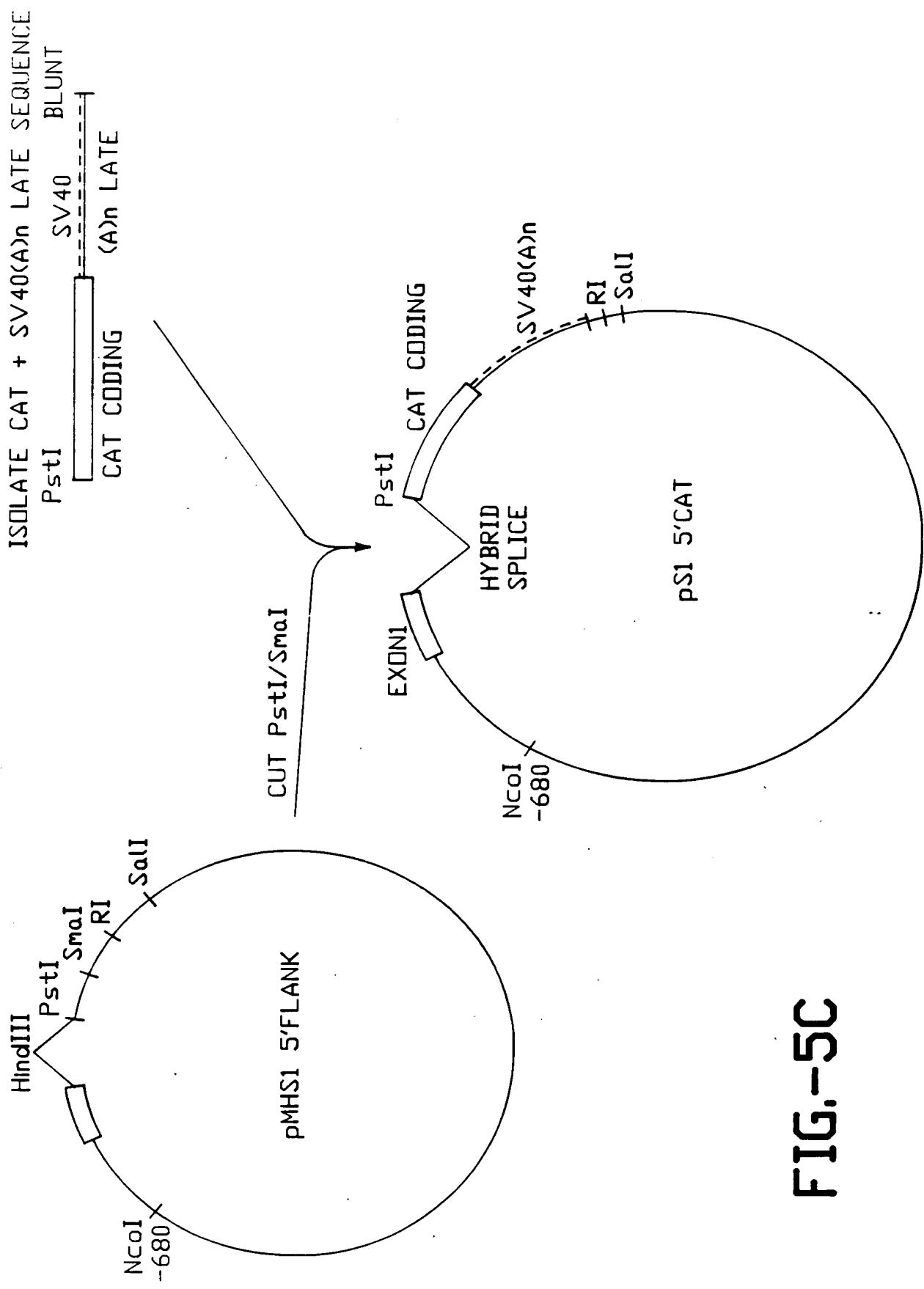


FIG.-5C

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NAME pMH-1

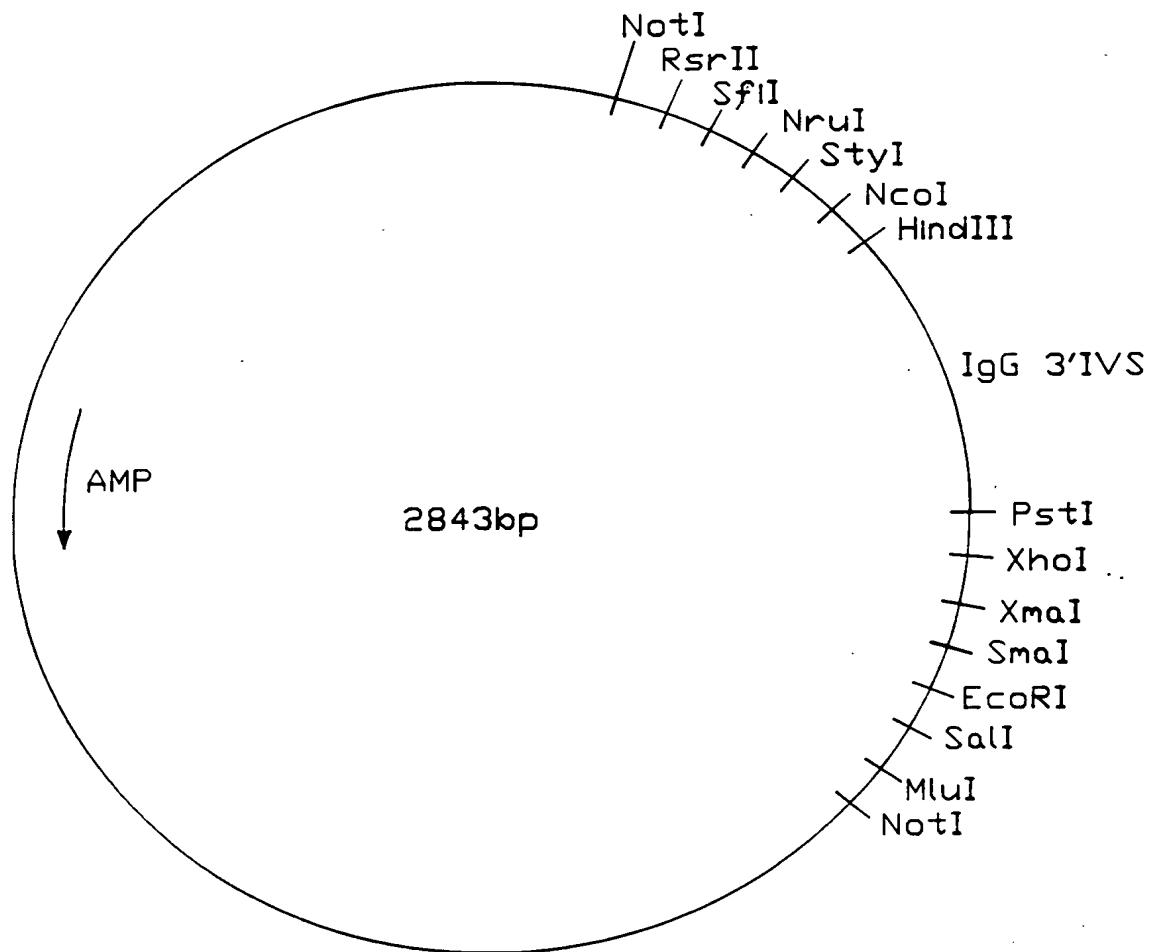


FIG.-6

- PLASMID [pMH3'E10] CONTAINS ~ 8.5Kb EcoRI FRAGMENT OF THE 3'-END OF α S1 CASEIN. pMH-1 WAS CUT W/EcoRI + LIGATED TO THIS FRAGMENT

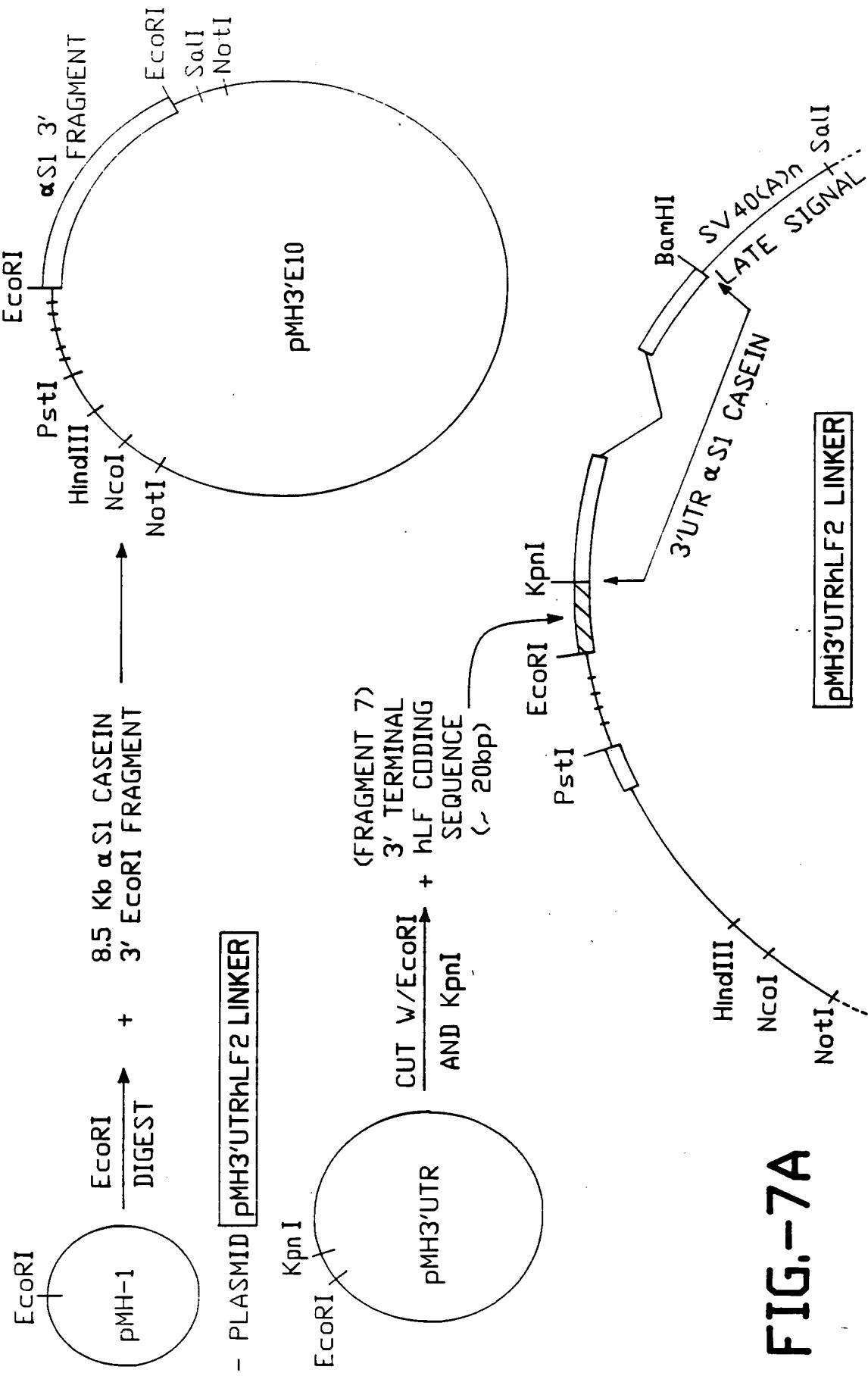
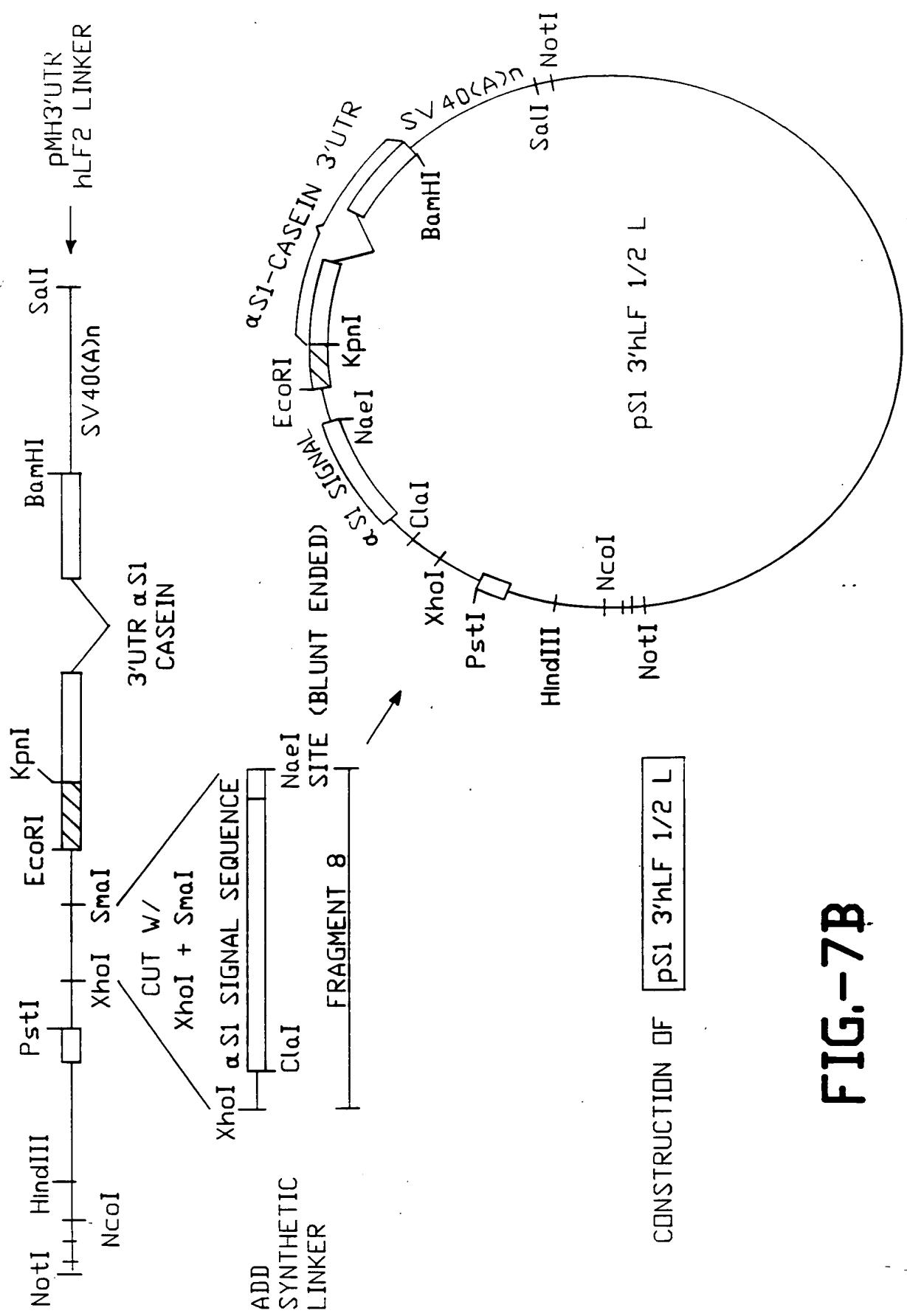
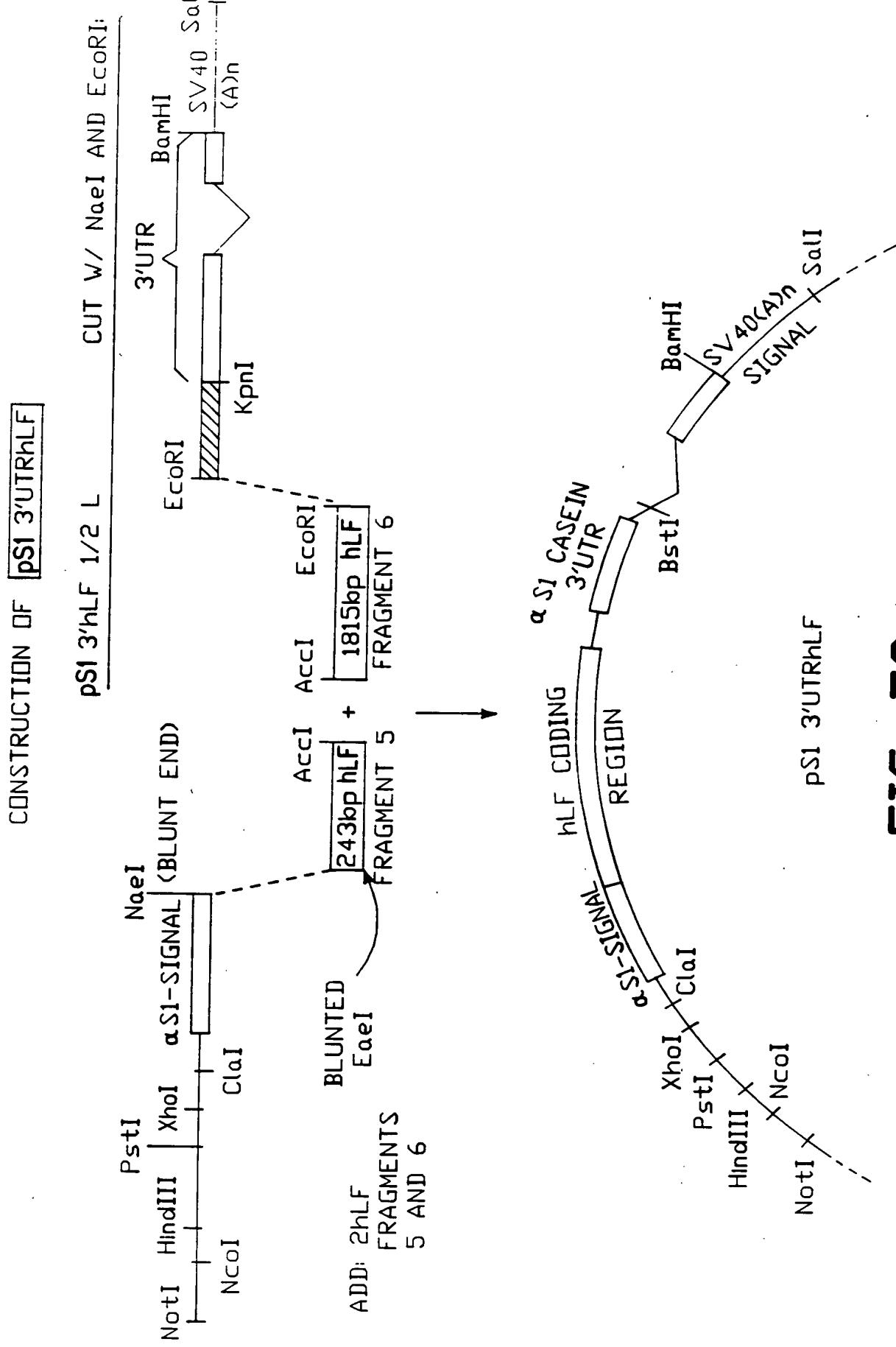
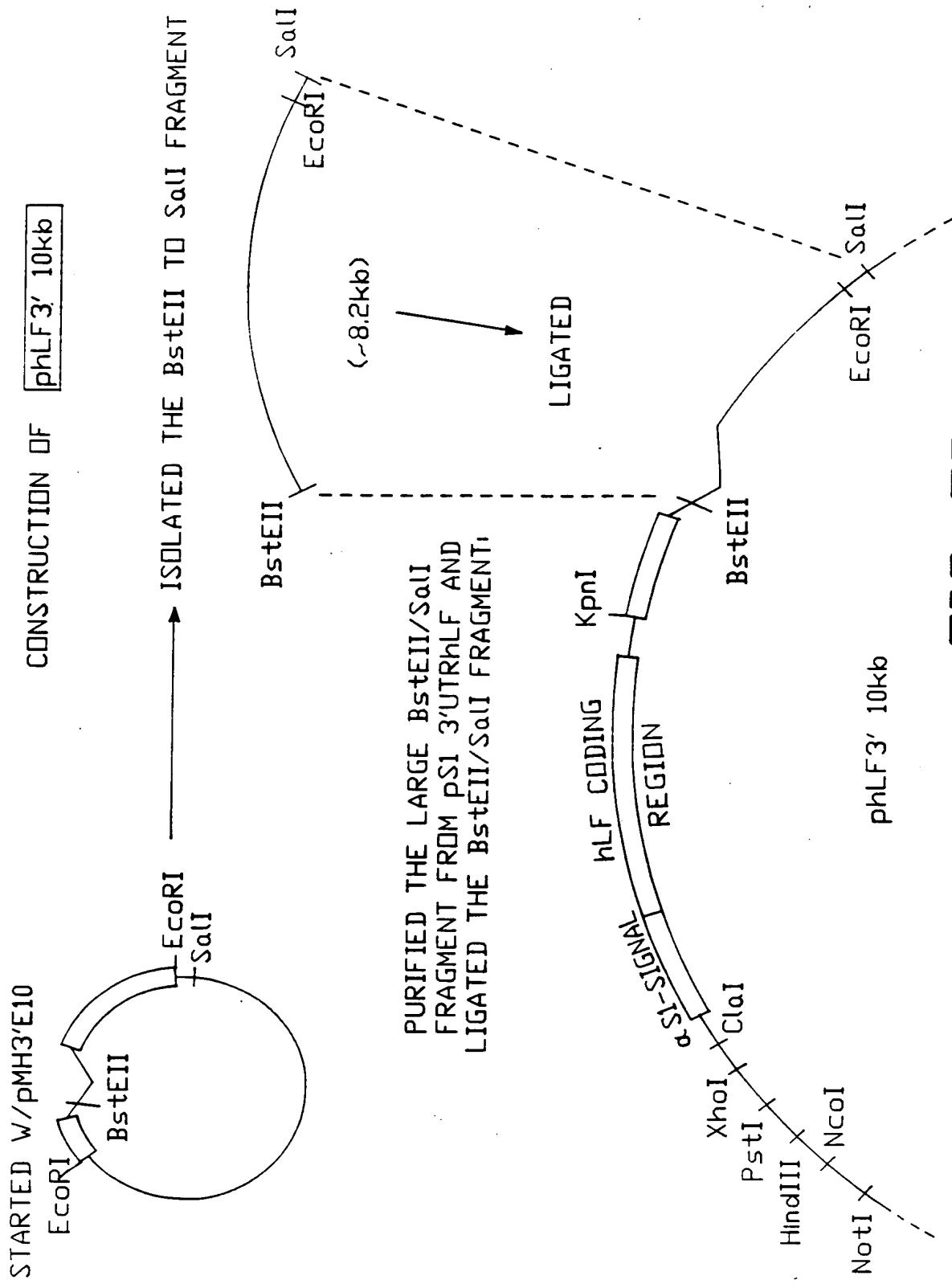


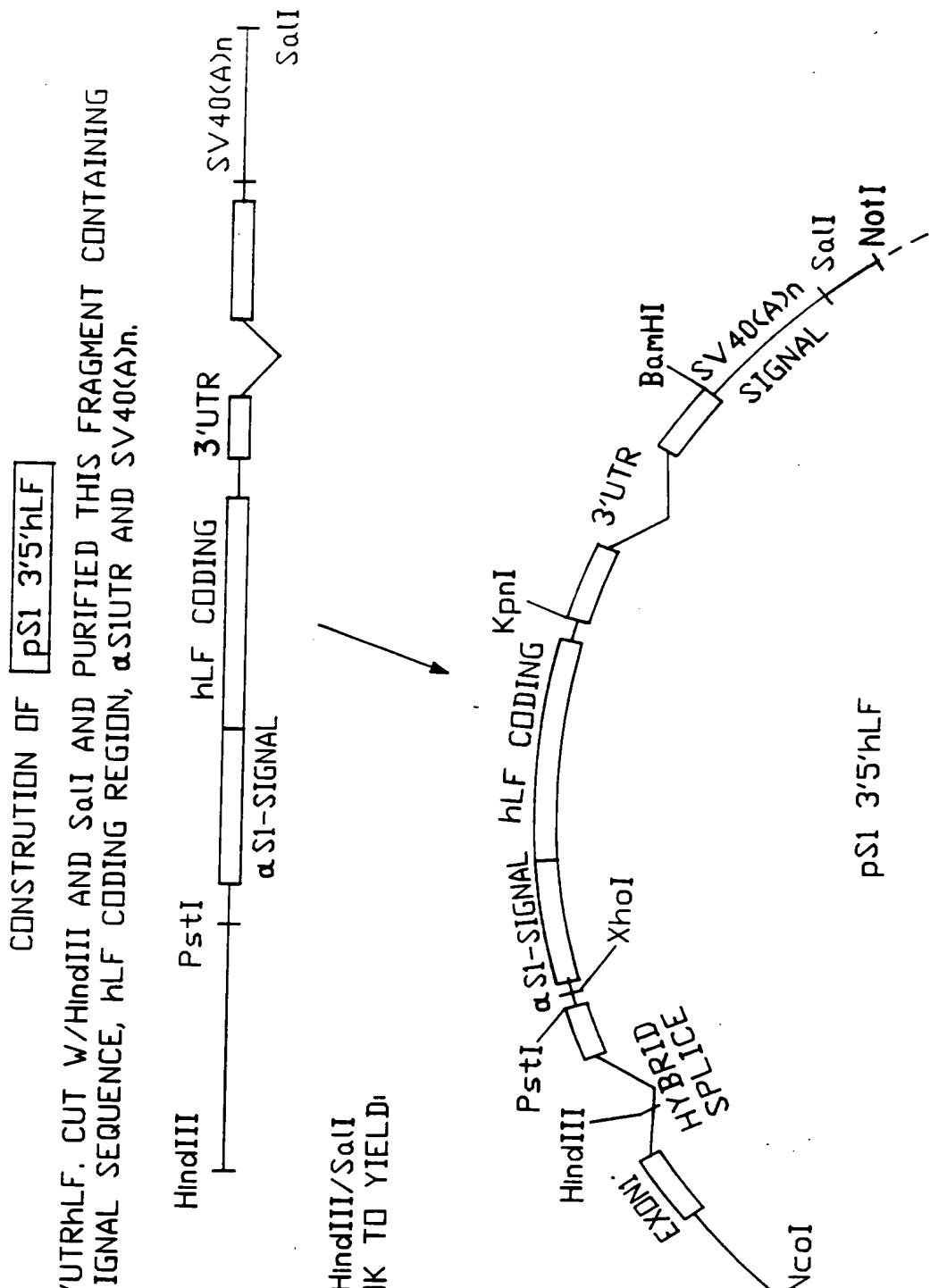
FIG.-7A

**FIG.-7B**

**FIG.-7C**

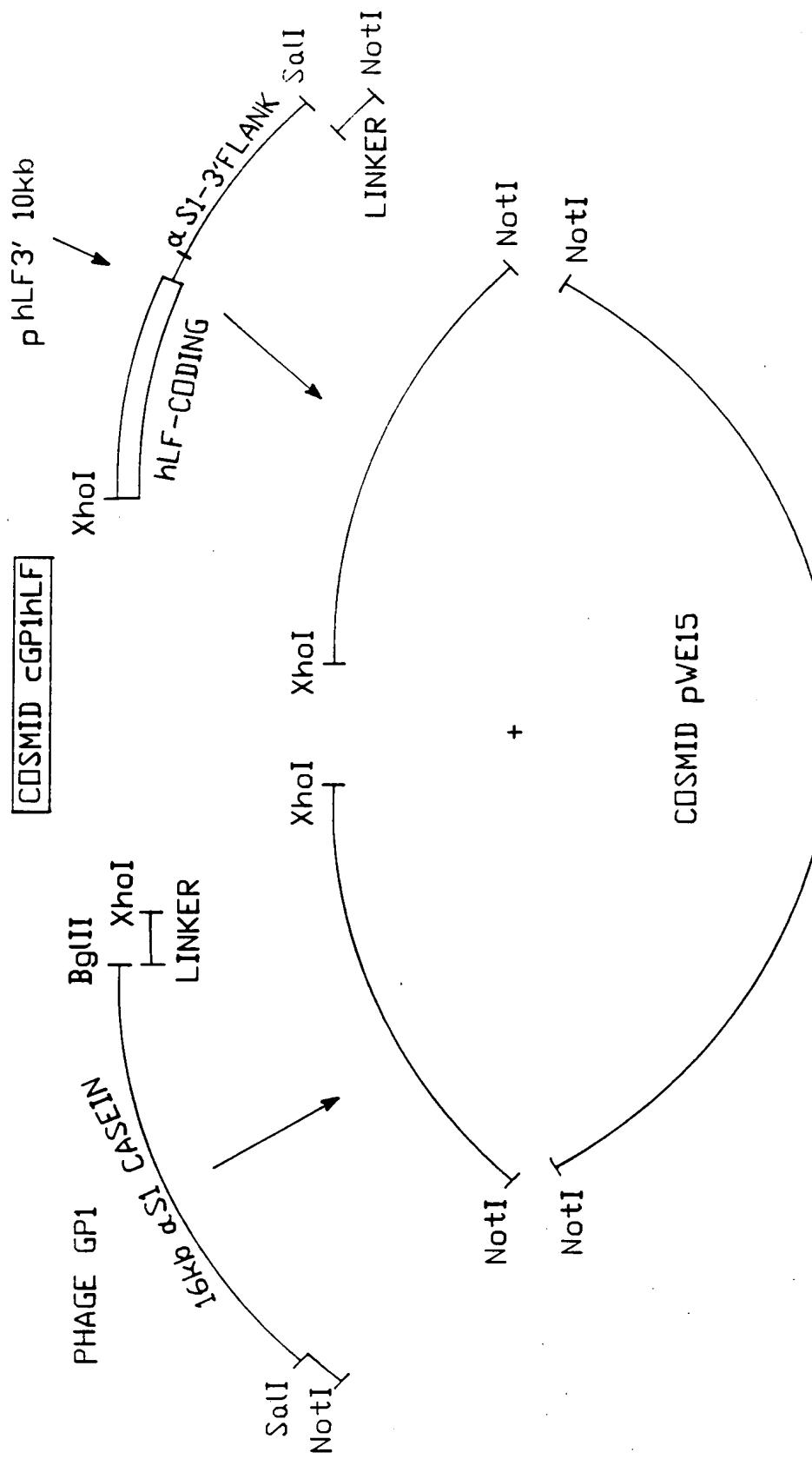
**FIG.-7D**

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NOTE: **pS1 5'hLF** WAS MADE BY CUTTING **pS1 3'5'hLF** W/KpnI AND BamHI, FOLLOWED BY BLUNTING THE ENDS AND RELIGATING. THIS ELIMINATES THE SPLICED 3'UTR REGION.

FIG.-7E



3 WAY LIGATION. THE DNA FROM THIS COSMID IS PREPARED BY CUTTING WITH NotI AND PURIFYING THE EXPRESSION SEQUENCE PRIOR TO MICROINJECTION.

FIG.-7F

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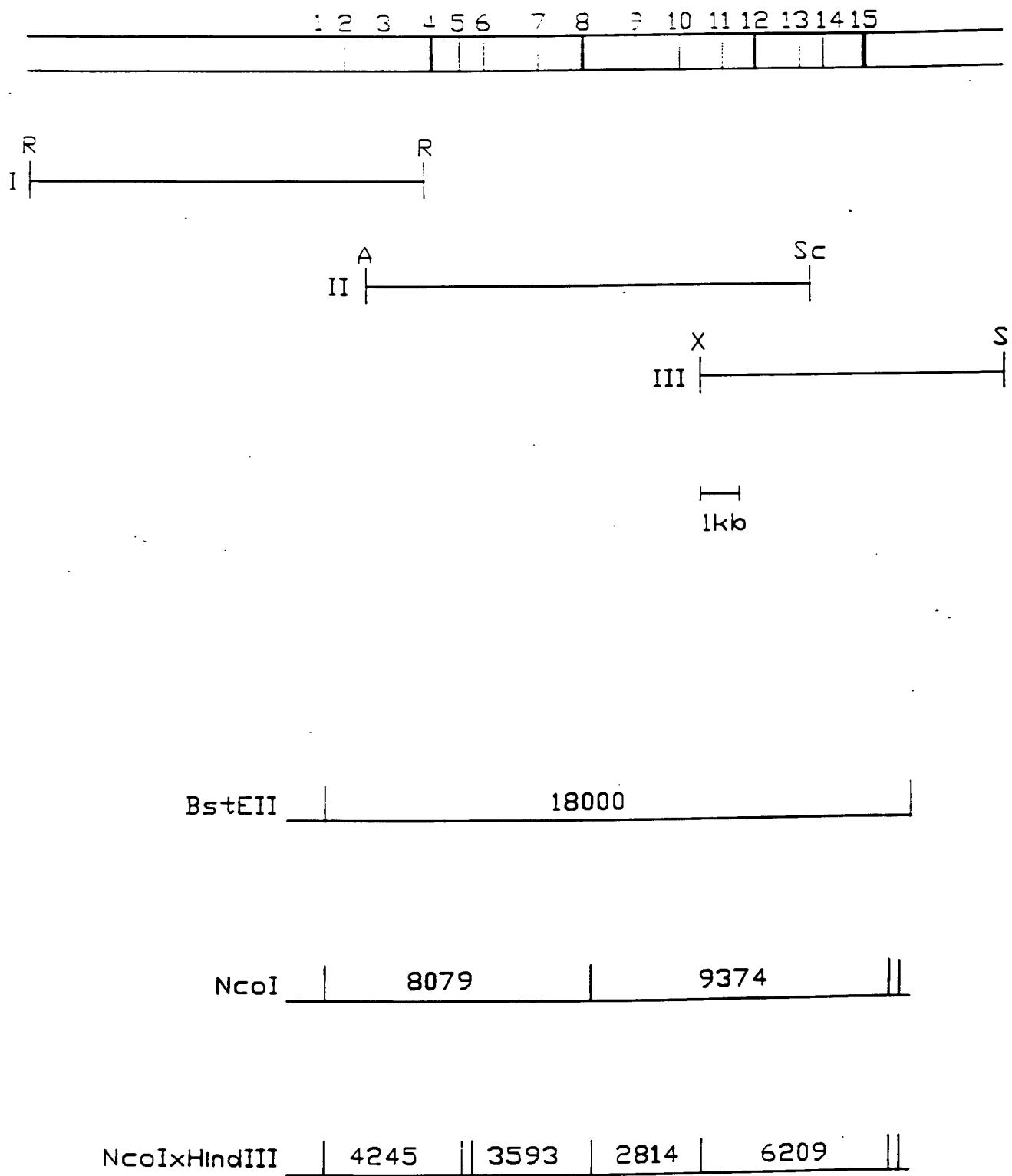
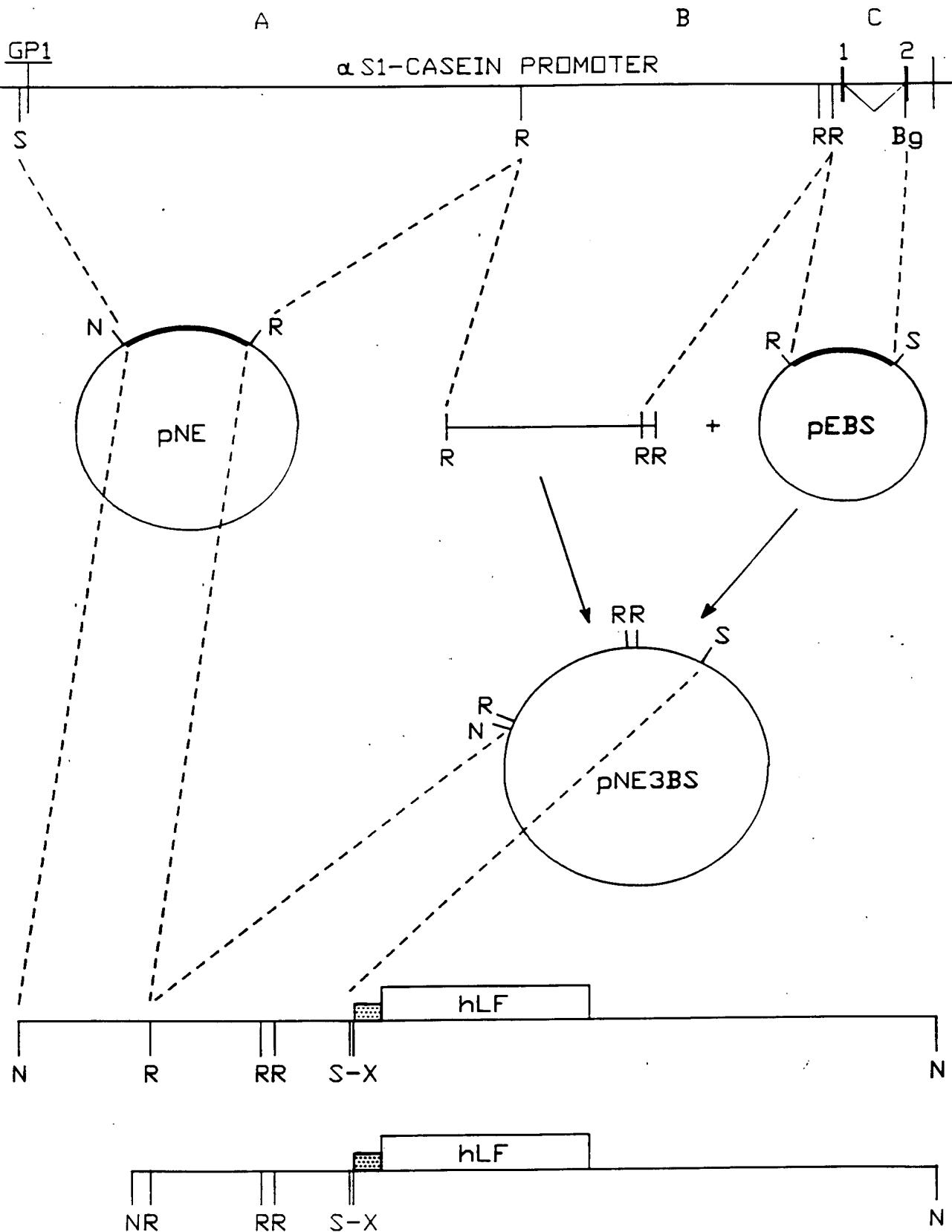
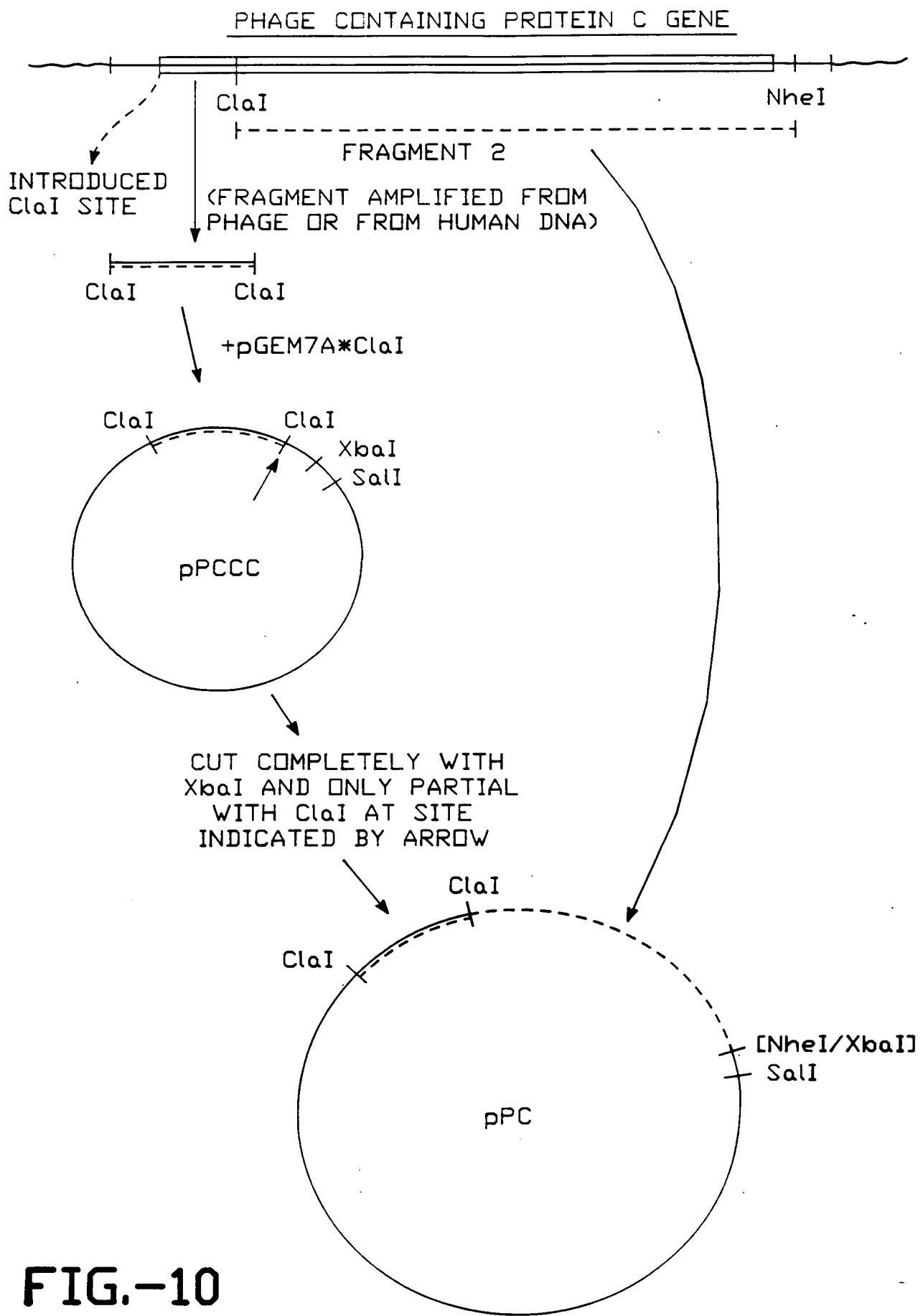


FIG.-8



S=Sall I R=EcoRI Bg=BglII N=NotI X=XbaI

FIG.-9



5'- ATCACCTTGA TCATCAACCC AGCTTGCTGC TTCTTCCCAG
TCTTGGGTTC AAG gttattatgt tacataaac aaaatttcta tgat~~tttct~~ ctgttcatac
~~tttcatatctt~~ cactaataacg cagttgtaaac ~~ttttctatgt~~ gattgcagg ~~attggtaat~~ tcctatgata
tactgttagc aatgttgagg tgtggcaggc ttgagatctg gccatacact tgagtacaa tgacatccac
~~tttgccat~~ tccacatcg GTGTCCACTC CCAGGTCCAA CTGCAG -3'

FIG.-11

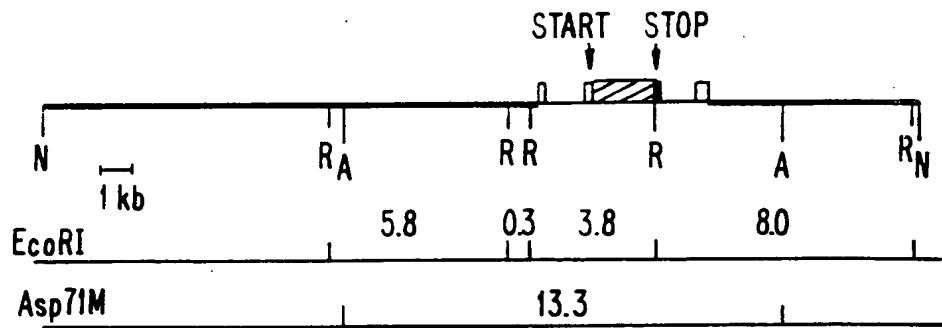


FIG. 12A.

COSMIDS CONTAINING ENTIRE hLF GENE (NOT DRAWN TO SCALE)

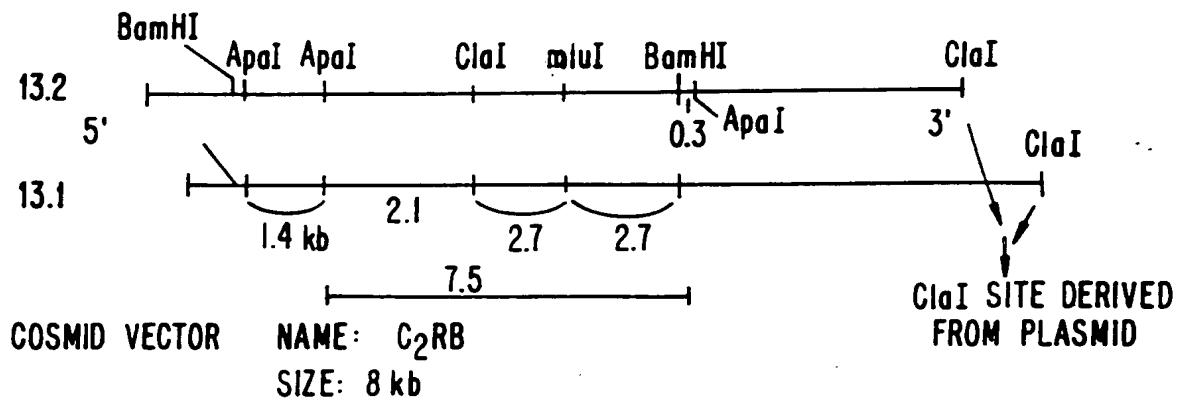


FIG. 13.

9 kb Bam HI hLF FRAGMENT IN pUC19

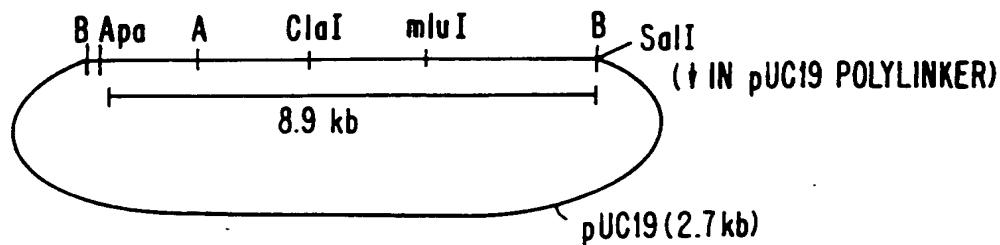


FIG. 14.

08/154019

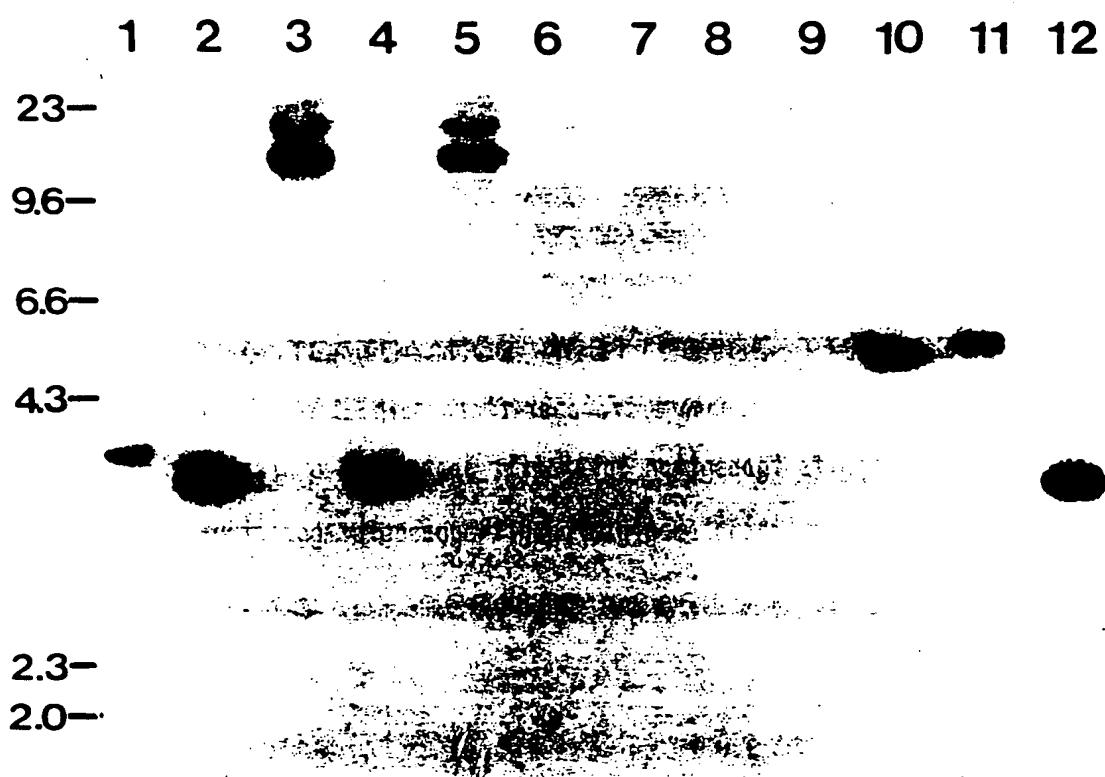
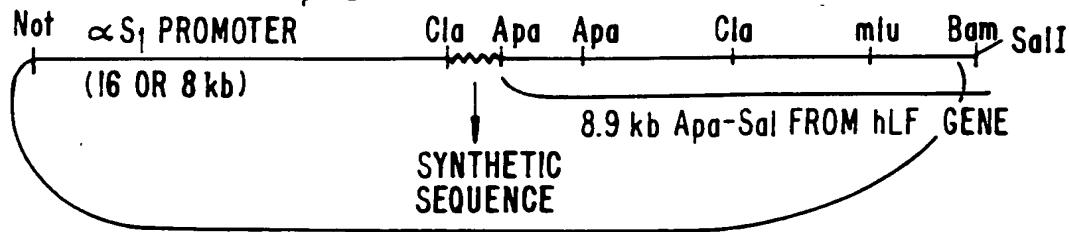


FIG. 12B.

LIGATION PRODUCT OF p16kbCS AND SYNTHETIC SEQ. (Cla-Apa)+ hLF FRAGMENT
(p8kbCS)



CLONING VECTOR: pkUN (4 kb)

CONSTRUCT NAME: 8 hLF gen 9k, OR 16 hLF gen 9k

FIG. 15A.

STRUCTURE OF *Cla*I-*Apa*I SYNTHETIC SEQUENCE

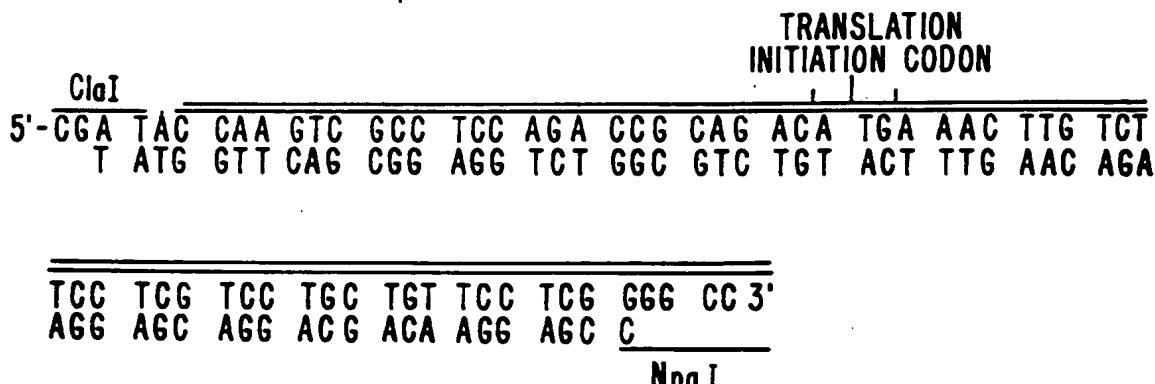
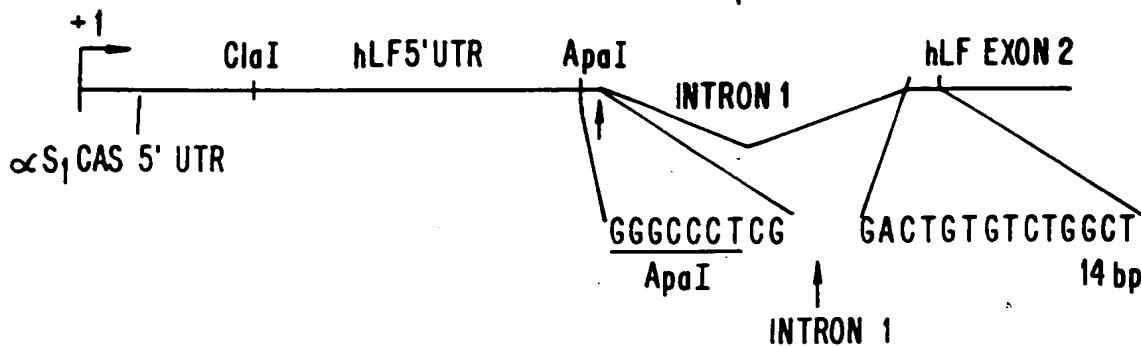


FIG. 15B.

+1: TRANSCRIPTION INITIATION SITE OF BOVINE αS_1 -CASEIN GENE



STRUCTURE OF REGION CONTAINING EXON 1 (HYBRID αS_1 -CASEIN/hLF EXON) AND PART OF EXON 2 OF THE GENOMIC hLF CONSTRUCTS DEPICTED IN FIGS. 15A THROUGH 17.

FIG. 15C.

08/15/4019

COINJECTION

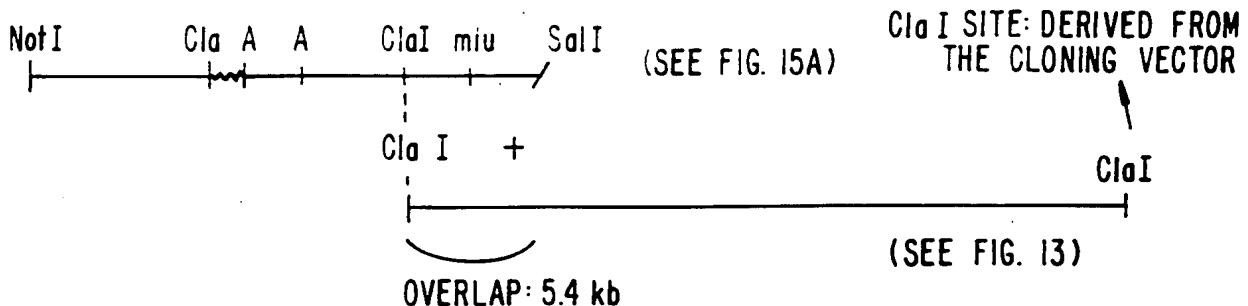


FIG. 16.

GENERATION OF 8hLF GENE

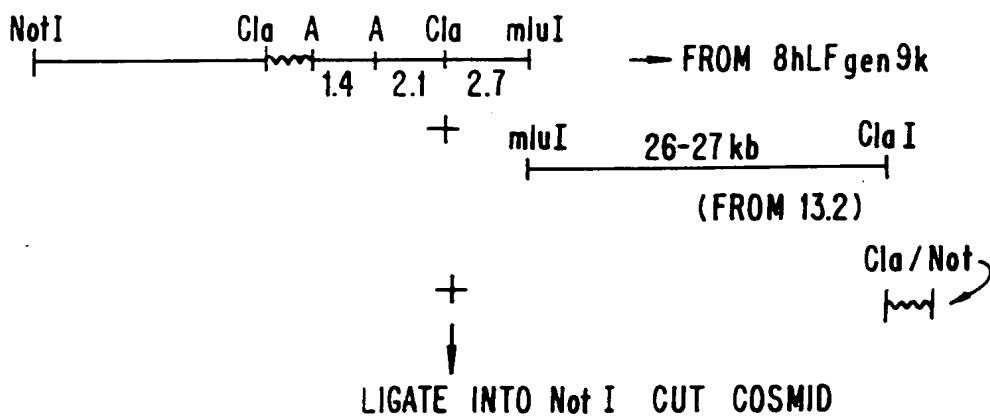
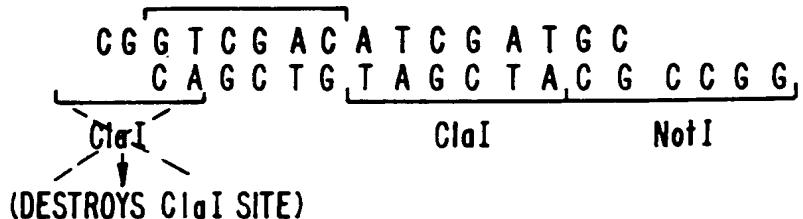
Cl_a/Not LINKER: SalI

FIG. 17.

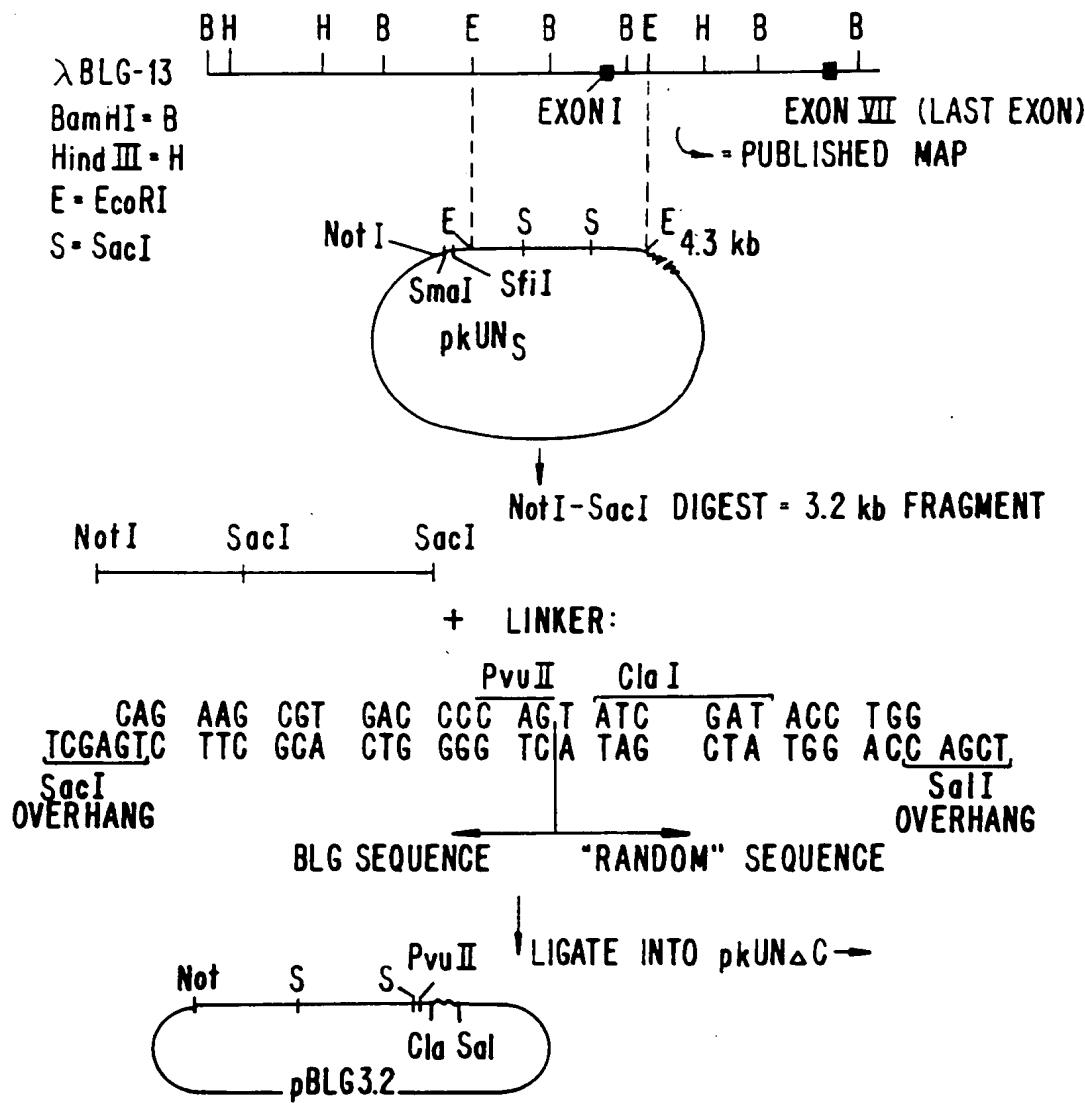


FIG. 18.

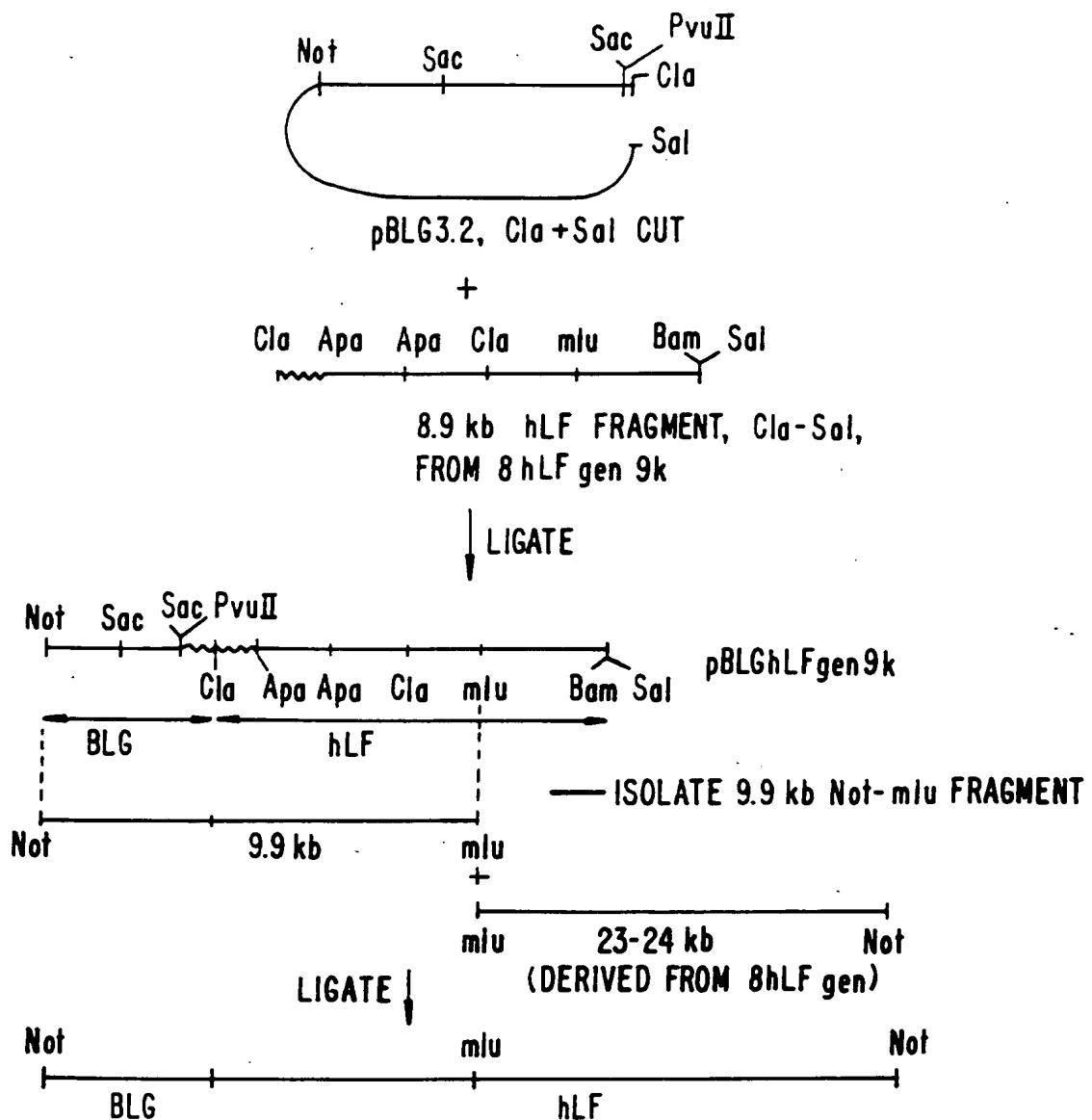


FIG. 19.

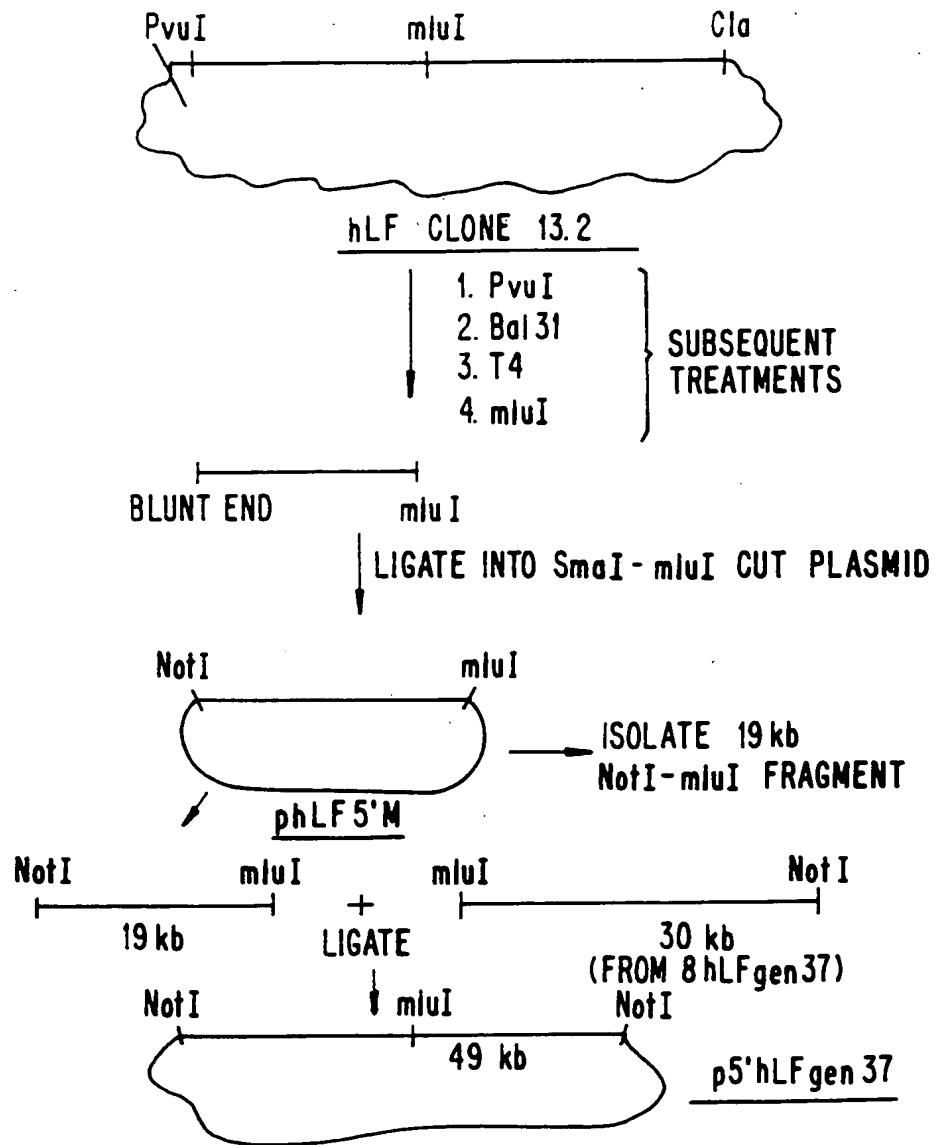


FIG. 20.

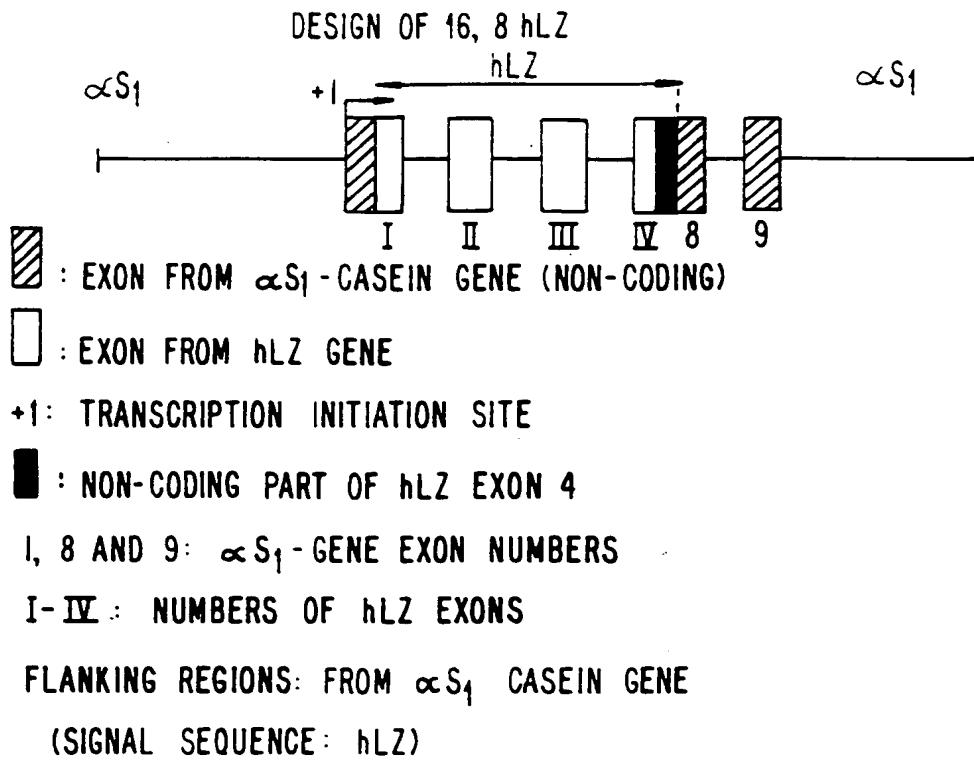


FIG. 21.

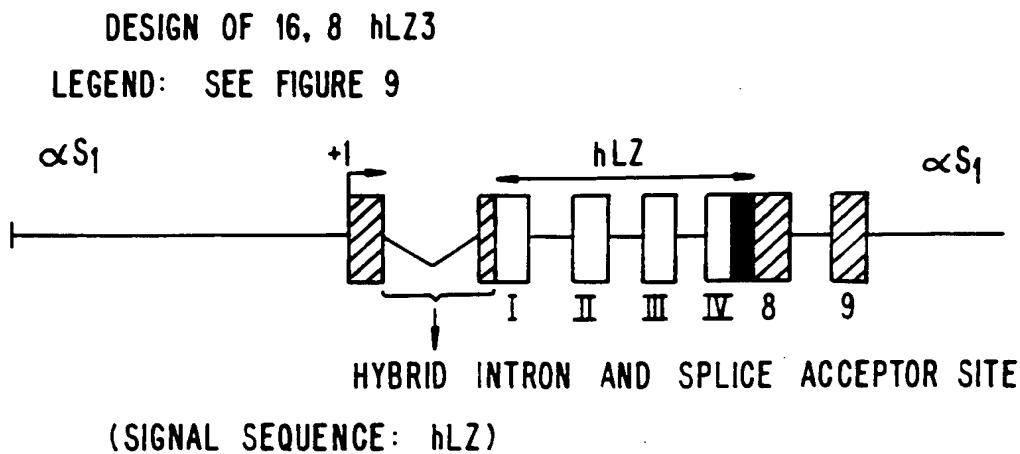
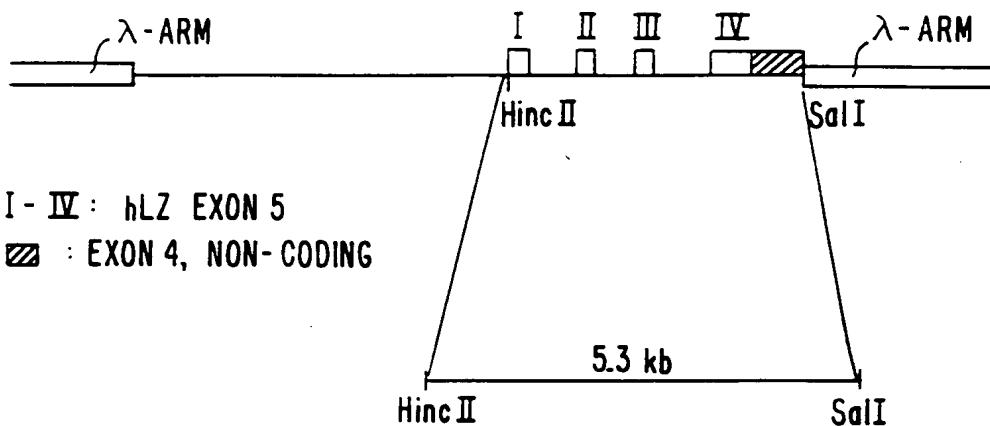


FIG. 22.

λ7.2.1

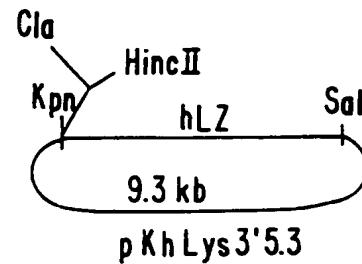


PRIMERS:

+3: 5'UTR hLZ

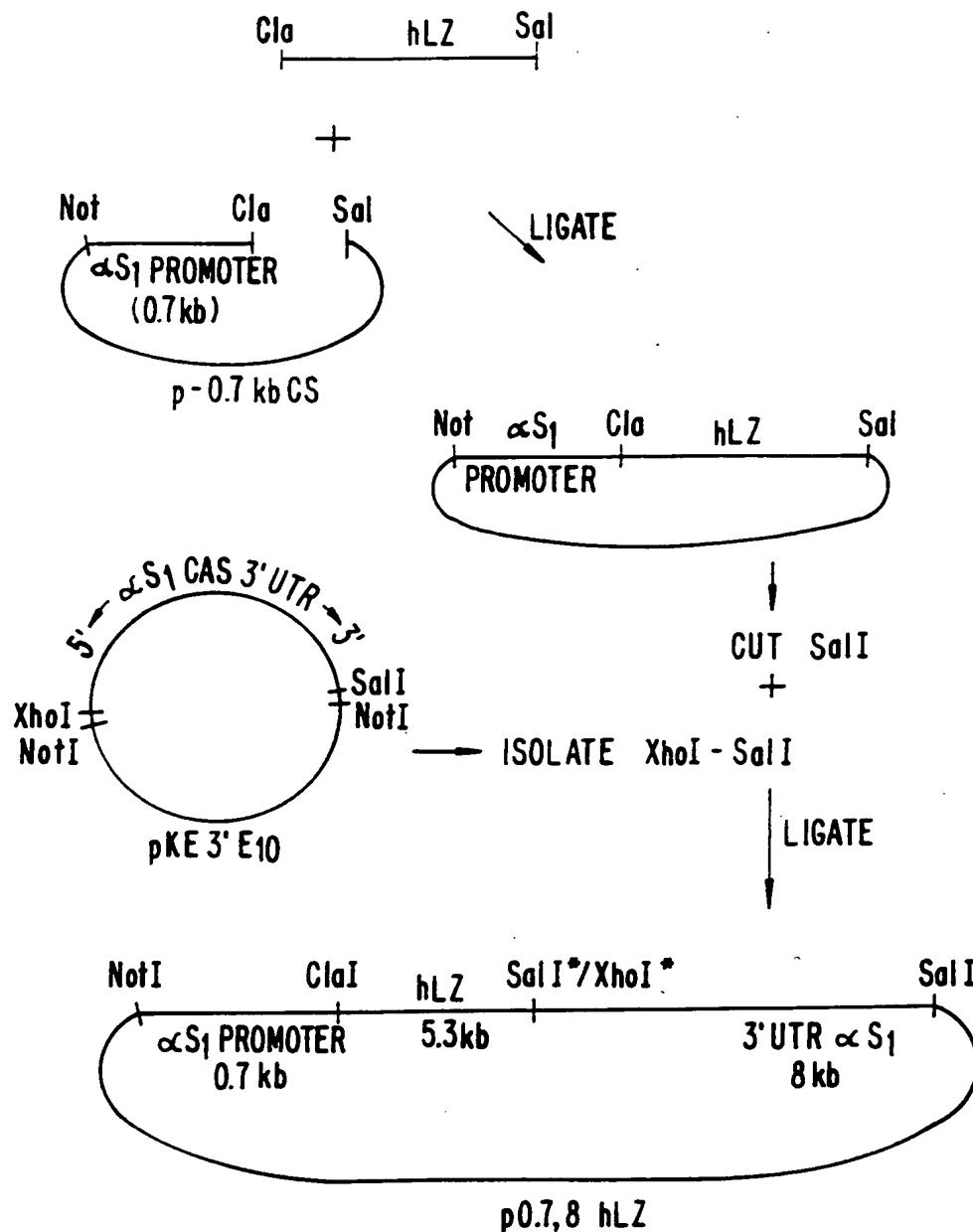
CAT G G T A G C T A G G G A T C G T G A G A C T G G A T C G T C A G
 ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓
KpnI Cla I Hinc II

LIGATE 5.3 HincIII
Kpn - HincII



Cla Sal

FIG. 23A.

**FIG. 23B.**

LINKER S₁/S₂

SalI^{*}- NotI - SalI⁺

• DESTROYED SITE

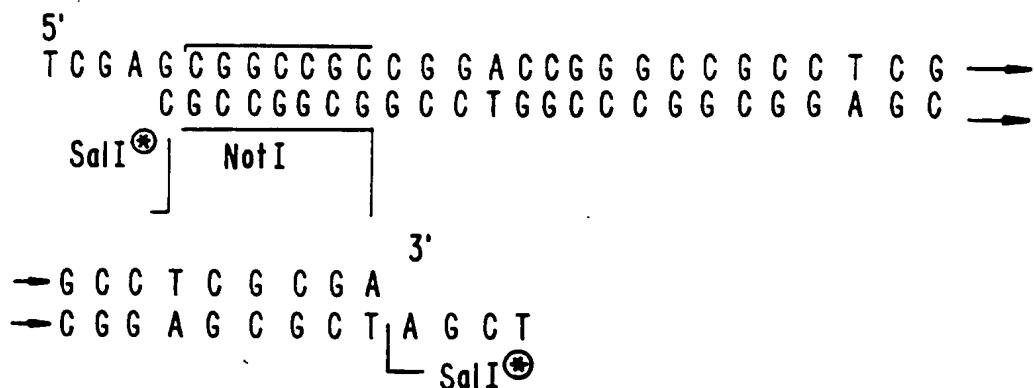


FIG. 23C.

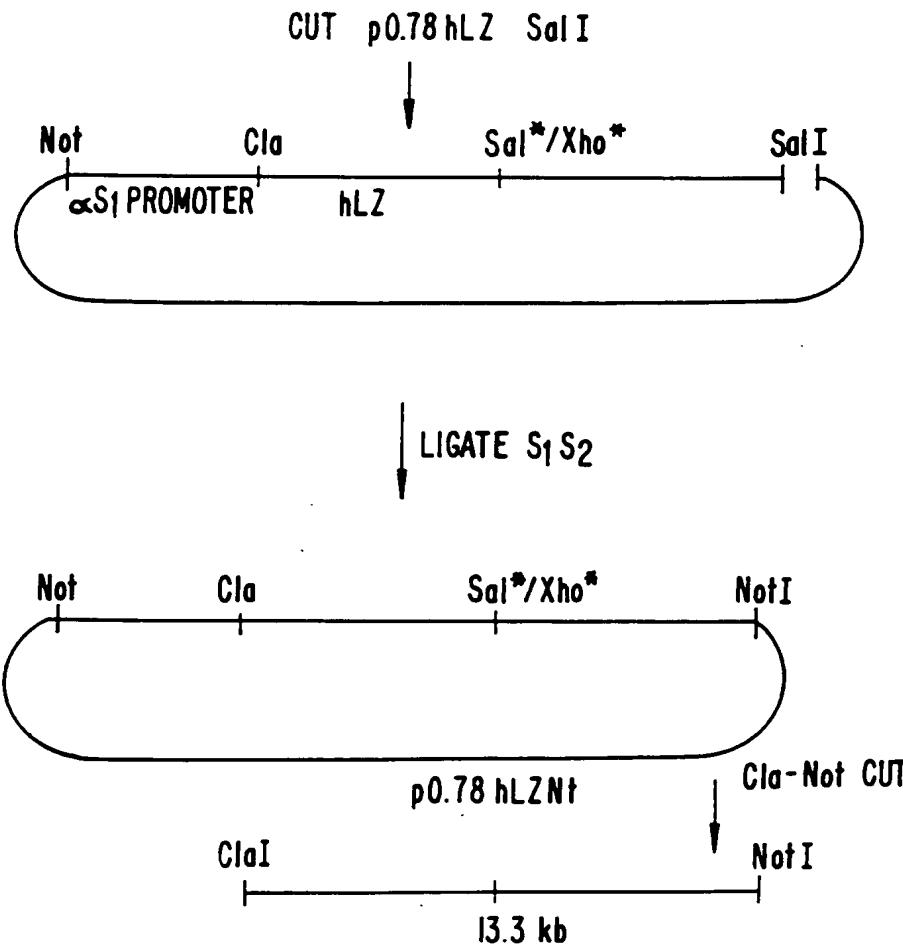


FIG. 23D.

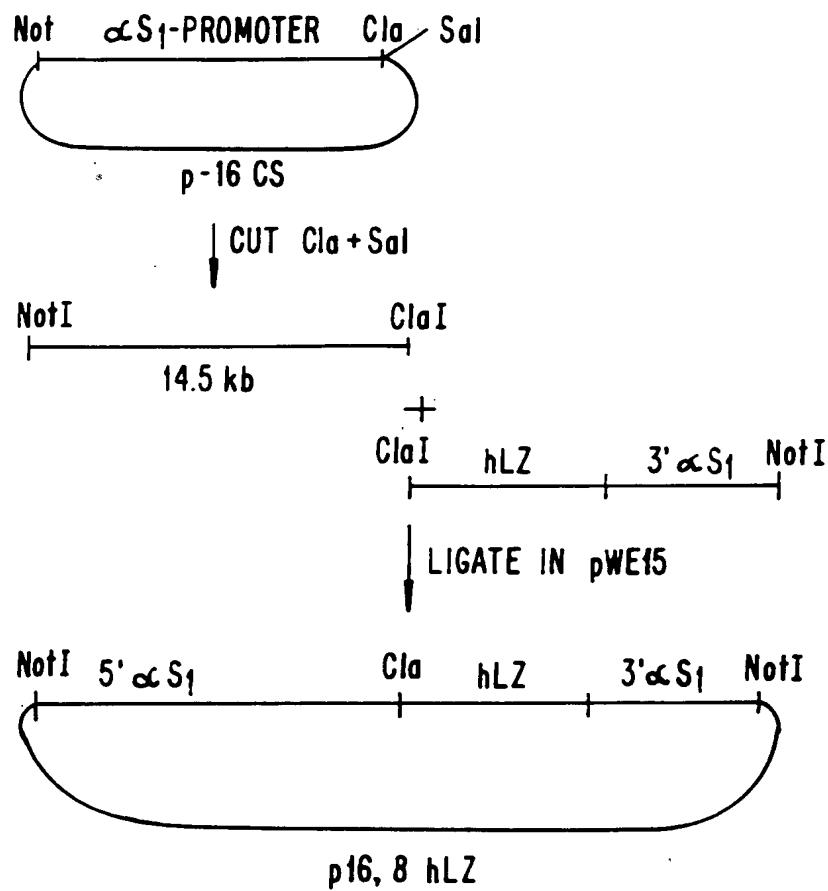


FIG. 23E.

BOVINE 2 GGAAGTGCCTGGAGA...TTAAAATGTGAGAGTGGAGT...GGAGGTTG 44
 SHEEP 84 GGAAGTGTCTGGGAGATTAAAATGTGAGAGGCGGGAGGTGGGAGGTGG 133
 45 GGTCCCTGTAGGCCTTCCCACGTGCTCACGGAGCCCTAGTGCTAC 94
 134 GGCCCTGTGGGCTGCCATCCCACGTGCCTGCATTAGCCCCAGTGCTGC 183
 95 TCAGTCATGCCCGCAGCAGGGTCAGGTCACTTCCCACCTGGGGT 144
 184 TCAGCCGTGCCCCGCCAGGGTCAGGTCACTTCCCCT. GGGGT 232
 145 TATTATGACTGTTGTCATTGTTGCCATTGGCATTTGCTACCCTAACGGGC 194
 233 TATTATGACTCTTGTCATTGCCATTGGCATTTGCTACCCTAACGGGC 282
 195 AGCGGGTGCCTGCAGAGCCCTCGATACTGACCAGGTTCCCCCTGGAGC 244
 283 AGCAGGTGCTTGCAAGAGCCCTCGATACCGACCAGG.TCCTCCCTCGGAGC 331
 245 TCGACCTGAACCCATGTCACCCCTGCCAGCCTGCAGAGGGTGGGTGA 294
 332 TCGACCTGAACCCATGTCACCCCTGCCAGCCTGCAGAGGGTGGGTGA 381
 295 CTGCAGAGATCCCTTACCCAAGGCCACAGTCACATGGTTGGAGGAGAT 344
 382 CTGCAGAGATCCCTCACCCAAGGCCACGGTCACATGGTTGGAGGAGCT 431
 345 GGTGCCAAGGCAGAACGCCACCCCTCCA.GAACACACCTGCCAGTGCTG 393
 432 GGTGCCAAGGCAGAGGCCACCCCTCAGGACACACCTGTCCCCAGTGCTG 481
 394 GCTCTGACCTGTCCTGTCATAAGAGGCTGACCCAGAAGTGGCTGGCG 443
 482 GCTCTGACCTGTCCTGTCATAAGAGGCTGACCCGGAAAGTGGCTGGCA 531
 444 CTGGCAGCCAGCCTGGACCCAGAGCCTGGACACCC.CCTGCCCCCCACT 492
 532 CTGGCAGCCAGCCTGGACCCAGAGTCCAGACACCCACCTGTGCCCGCT 581
 493 TCTGGGGCGTACCAAGAACCGTCCAGGCCAGA..GGGCCTTCCTGCTT 540
 582 TCTGGGGTC.TACCAAGAACCGTCTAGGCCAGAGGGGACTTCCTGCTT 630
 541 GGCTCGAATGGAAGAACGCCCTCTATTGTCCTTCGTAAGAAGCAACC 590
 631 GGCTTGGATGGAAGAACGCCCTCTATTGTCCTTCGTAAGAAGCAACC 679
 591 CCAGGGCCAAGGATAGGCCAGGGGATTGGGGAAACCGCGTGGCT.CC 639
 680 CGGGGGCCTGAGGATGAGCCAAGTGGGATTCCGGGAACCGCGTGGCTGG 729
 640 GGGCGGGCCCCGGCTGGCTGGCTGGC..CCTCCTCCTGTATAAGGCCCG 687
 730 GGCCAGCCCCGGCTGGCTGGCTGGCATGCGCCTCCTGTATAAGGCCCA 779

FIG. 24

688 AGCCCG. CTGTCTAGCCTCCACTCCCTGCAGAGCTCAGAAGCGTGACC 736
 ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
 780 AGCCTGCCCTGCTCTAGCCTCCACTCCCTGCAGAGCTCAGAAGCACGACC 829
 ||||| ||||| |||||
 737 CCAGCTGCAGCCATGAAGTGCCTCTGCTTGCCTGGCCCTGGCCCTCGC 780
 |||||
 830 CCAGCTGCAGCCATGAAGTGCCTCTGCTTGCCTGGCCCTGGCCCTCGC 879
 |||||
 781 CTGTGGCGCCAGGCCCTCATCGTCACC 808
 |||||
 880 CTGTGGCGTCCAGGCCATCATCGTCACC 907

* TRANSLATION INITIATION CODON

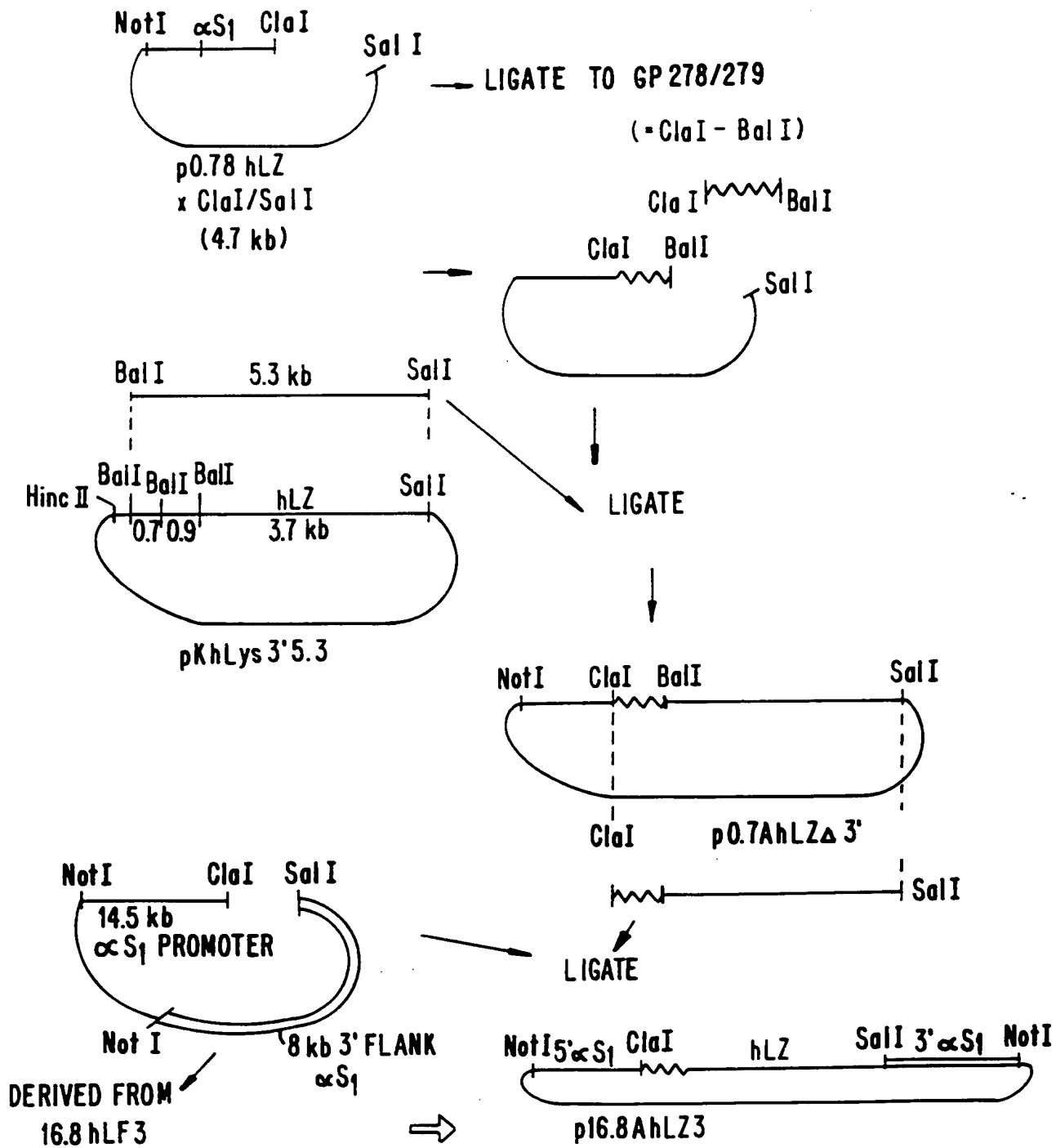
FIG. 24 [continued]

LINKER GP 278/279

TRANSLATION START SITE (αS_1 SIGNAL SEQUENCE)
ClaI ↓
 CGATAACCATGAAACTTCTTATCCTCACCTGTCTTGTGGCTGTTGCTTTG
 |
 TATT — ETC.

αS_1 — hLZ SEQUENCE BalI
 —CCAAGGTCTTGAAGGTGTGAGTTGC
 ETC.—AAC

FIG. 25.

CONSTRUCTION OF 16.8 A hLZ3:

CONSTRUCTION OF 16 A hLZ3:

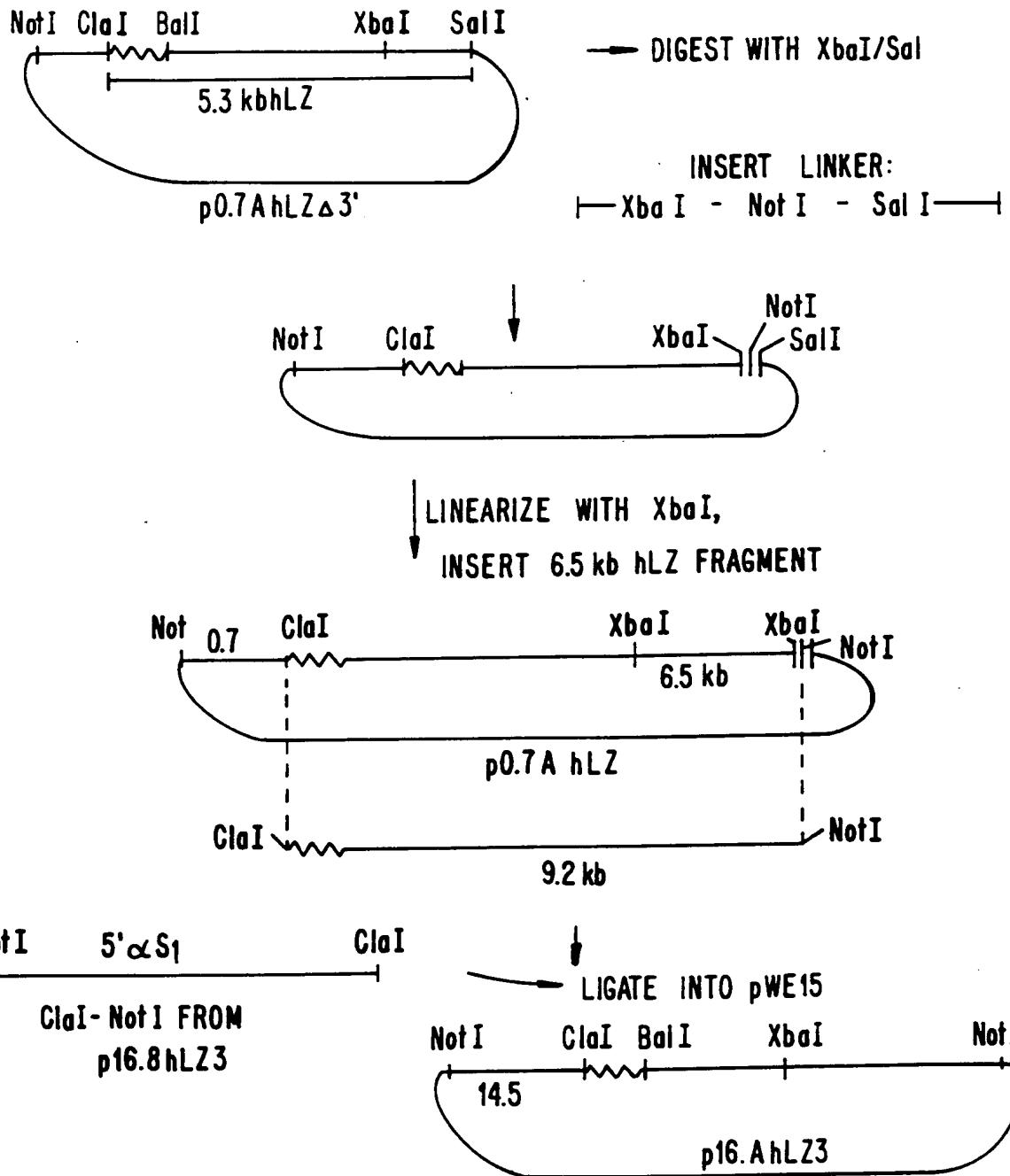


FIG. 27.